Effects of Low Doses of Quinpirole on Production of 50 kHz Vocalizations in Wistar Rats

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Rats emit two distinct types of ultrasonic vocalizations in adulthood: 22 kHz (aversive situation), and 50 kHz calls (appetitive situation). The present project is focussed on pharmacological studies of 50 kHz vocalizations. The 50 kHz calls are elicited from dopaminergic activation in the mesolimbic pathway and are emitted in such appetitive situations as social contact(s), sexual encounters, food reward, etc. Eighty-five male rats were stereotaxically implanted with bilateral guide cannulae in the nucleus accumbens shell (A= 9.7, L= 1.2, V= 6.7). Quinpirole, a D_2/D_3 dopaminergic agonist, was injected in low doses to the nucleus accumbens shell in an attempt to elicit 50 kHz vocalizations. A dose response was obtained for the low dose range of quinpirole for six doses: 0.025 μg, 0.06 μg, 0.12 μg, 0.25 μg, 0.5 μg, and 1.0 μg. It was found that only application of the 0.25 μg dose of quinpirole and the 7 μg dose of amphetamine (positive control) significantly increased the total number of 50 kHz calls ($p < 0.006$ and $p < 0.004$ respectively); and particularly significantly increased the frequency modulated type of these calls ($p < 0.01$, and $p < 0.006$ respectively). In a double injection procedure, the dose of 0.25 μg quinpirole was antagonized with raclopride (D_2 antagonist) or U99194A maleate (D_3 antagonist) in an attempt to antagonize the response. The 0.25 μg dose of quinpirole was successfully antagonized by pre-treatment with an equimolar dose of U99194A maleate ($p < 0.008$) but not with raclopride. The 7 μg amphetamine response was also antagonized with an equimolar dose of raclopride. Based on these results, it seems that low doses of quinpirole, particularly the 0.25 μg dose, are capable of increasing 50 kHz vocalizations in rats and do so by activation of the D_3 dopamine receptor. This is not a biphasic response as seen with locomotor studies. Also noteworthy is the increase in frequency modulated 50 kHz calls elicited by the 0.25 μg dose of quinpirole indicating a possible increase in positive affect.

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USV(s) = ultrasonic vocalization(s)

DA = dopamine

CNS = central nervous system

VTA = ventral tegmental area

MAO = monoamine oxidase

OCD = obsessive compulsive disorder

DAT = dopamine transporter

PD = Parkinson's Disease

i.p. = intraperitoneal (injection)

s.c. = subcutaneous (injection)

Introduction

Animal Communication

Animal communication mechanisms have been well studied over the years and it is a complex subject due to the mostly species-specific nature of animal communication (Portfors, 2007). What remains consistent across the myriad of ways in which animals communicate is the concept of the signal, whatever modality that may be. Signals have a communicative purpose, i.e., are of adaptive value to the animal sending and/or receiving the signal (Brudzynski, 2005). Animals need to be able to communicate quickly and reliably regarding basic survival of the species, i.e., approaching predators, or in social situations, i.e., establishment of hierarchal systems (Bradbury & Vehrencamp, 1998; Brudzynski, 2005). This type of information may be termed "biologically significant stimuli or cues" and allows the receiver of the relevant information to better predict their current environment and to act accordingly (Brudzynski, 2005).

One of the most effective forms of communication (with respect to the communicative speed between sender and receiver) across anurans, avians, and mammals is the method of sound production to convey information (Bradbury & Vehrencamp, 1998). The term used for this type of communication across species is bioacoustic signalling. In mammals, vocal sound production is accomplished by a combination of respiratory, laryngeal, and supra-laryngeal control and by adjusting that control to homeostatic needs allowing for a variety of sounds to be produced and emitted (Jürgens & Ploog, 1981). These sounds can be produced in the human audible range (20 Hz – 20 kHz), and also in the ultrasonic range of frequency which is above 20 kHz (Sales & Pye, 1974; Brudzynski, 2005, Portfors, 2007).

Rat Communication – Ultrasonic Vocalizations

Rats, like most other mammals, have anti-predator defensive adaptations to protect themselves from the large number of animals that prey on them. Although rats do communicate to other species through squeals in the 2 - 4 kHz range (in an attempt to ward off an attack), their main method of bioacoustic signalling within their own species is through ultrasonic vocalizations (Blanchard, et al., 1991; Brudzynski, 2005; Portfors, 2007; Brudzynski, 2009). These ultrasonic vocalizations (USVs) are emitted on the whistle principle: the rat makes an inspiration, the larynx is stabilized, and the vocal folds are slightly separated so as to produce sound in the ultrasonic range, in a similar way to that of a human whistle (Brudzynski, 2005; Brudzynski, 2009).

The USVs are a useful means of communication for rats as they have a nocturnal lifestyle and live in colonies and are very social animals (Blanchard, et al., 1991; Wöhr, & Schwarting, 2007; Brudzynski, 2005). Since most of their predatory species do not hear in the ultrasonic range, these calls likely go undetected while having a meaningful message for conspecifics (Blanchard, et al., 1991). However, the most important function the USVs appear to serve is in the non-defensive social domain (Brudzynski, 2009). Rats are extremely gregarious animals and if given the choice, would rather spend time with conspecifics than in isolation or time spent with inanimate objects (Wöhr & Schwarting, 2007). Studies in which rats were de-vocalized prior to social meetings showed deficits in their social encounters and these deficits were able to be improved by the playback of natural USVs (White & Barfield, 1987, 1989, & 1990).

The ultrasonic calls used by rats have distinctive acoustic features that may offer a way of quantifying the magnitude of the sign meaning within that call (Portfors, 2007; Brudzynski,

2005). For example, the decibel level of the call could indicate the urgency of the message, much like a human raising their voice. Also, it is speculated that the higher the number of vocalizations emitted within a certain time period, the greater the magnitude of response in the receivers. USVs may encode only one meaning (monosemic calls), or they may convey more than one message to the recipient (polysemic calls). Biologically, it is more beneficial for rats to encode more than one meaning in their USV signals (Brudzynski, 2005). For instance, these calls may serve as a means of localization allowing the sender and receiver to assume proximity; they inform conspecifics of predators and aversive situations which promotes freezing behaviours or escape. Also, these USVs serve an emotional and social function allowing rats to communicate their overall state to conspecifics, and to organize social approach or avoidance (Brudzynski, 2005; Portfors, 2007; Burgdorf, et al., 2008). There are many possibilities pertaining to the function of these calls. As seen in many studies, emitted USVs evoke behavioural activation and a change in behaviour of the rat recipient. Behavioural responses indicate that these calls serve a communicative purpose and can elicit an appetitive or aversive response from the recipient even though the USVs differ depending on age, the subject's current state, and environmental factors (Wöhr, & Schwarting, 2007; Portfors, 2007; Burgdorf, et al., 2008; Wöhr et al., 2008; Brudzynski, 2009). There are three distinct types of ultrasonic vocalization emitted by rats: pup isolation calls, 22 kHz calls, and 50 kHz calls.

Rat pup isolation calls

The first of the ultrasonic vocalizations are observed as pup isolation calls. These calls vary in frequency and duration and have a drastic fluctuation in sonographic structure (Sales & Pye, 1974; Sales, 1979a; Brudzynski, 2005; Portfors, 2007). Calls may vary from 20-160 ms in

duration, 10-120 kHz in frequency range, and may have a bandwidth anywhere from 2 to 180 kHz (Brudzynski, et al., 1999). Even though there is great variation in call frequency, studies have shown that the frequency sweeps (U or hump shaped) within these calls are most likely where the message is encoded (Sales, 1979a; Brudzynski, et al., 1999; Hashimoto, et al., 2004; Brudzynski, 2005; Portfors, 2007), as well as the total number of these calls emitted by pups (Sales, 1979a; Brunelli, et al., 1994). The message within these calls is directed toward the mother, dam (Sales, & Pye, 1974; Portfors, 2007). When pup isolation calls are emitted, retrieval behaviour in the dams as well as nest building activity is elicited. The number of calls emitted by the pups directly correlates to the intensity of the searching and retrieval behaviour in the dams (Sales, 1979a; Brunelli, et al., 1994). As to pups, survival is near impossible without the dam's care and attendance. These pup "distress" calls are extremely important to ensure that pups stay within the nest and that they receive the care and warmth they need to survive. Thus, these calls are critical for survival (Sales, 1979a; Brudzynski, 2005).

Isolation calls can be elicited by removing a pup from its litter and/or nest (Wöhr, & Schwarting, 2007; Brudzynski, 2005; 2009), which results in a drop in temperature, a lack of tactile stimulation, and nutritional state (Sales, 1979a), all of which cause the pups to "alert" their mother. The anatomical structures involved in the production of the rat pup isolation calls include the thalamus, hypothalamus, amygdala, nucleus accumbens, preoptic area, septum, and the anterior cingulate cortex projecting to the periaqueductal grey (Hofer, 1996). Indeed, the subcortical regions of the brain are responsible for the emission of isolation calls seen in rat pups as ablation of the cortex does not attenuate isolation calls when cold stimulation is applied to simulate removal from the litter (Middlemis-Brown, et al., 2005). The rat pup isolation calls are

only seen up to day 16-20 of age (Sales & Pye, 1974; Sales, 1979a; Kehoe, et al., 2001; Portfors, 2007), after which the two distinct types of adult USVs are observed: 22 kHz calls and 50 kHz calls.

22 kHz Vocalizations

One type of ultrasonic vocalization used by the adult rat is the 22 kHz call. These calls are emitted during aversive situations or in the anticipation of an aversive encounter: foot shock (Borta, et al., 2006; Portfors, 2007), presence of a predator (Blanchard, et al., 1991; Brudzynski, & Ociepa, 1992; Portfors, 2007), during the post-ejaculatory period (Barfield & Geyer, 1972; Lore, et al., 1976; Sales, 1979a; Blanchard et al., 1991; Van der Poel & Miczek, 1991; Brudzynski, & Ociepia, 1992), during inter-male aggression (Sales, 1979; Takeuchi, & Kawashima, 1986; Portfors, 2007), after startling noises (Kaltwasser, 1991; Portfors, 2007), rough handling (Brudzynski & Ociepa, 1992), or any situation that induces an overall negative affective state (Knutson, et al., 2002). These calls are also known as "alarm" calls because they can signal danger to the entire colony. It has been hypothesized that these calls signal internal states that are homologous to the human emotions of anxiety and sadness; and anxiolytics attenuate the 22 kHz call response (Wöhr, & Schwarting, 2007; Wöhr, et al., 2008). However, this anxiety and subsequent emission of 22 kHz calling is due to potential danger not the direct painful event itself (Blanchard et al., 1991; Van der Poel & Miczek, 1991; Jourdan, et al., 2002; Brudzynski, 2009). For example, rats responded with higher numbers of 22 kHz calls when a foot shock was unavoidable compared to the same intensity of shock, but avoidable. (Kikusui, et al., 2003; Borta, et al., 2006; Portfors, 2007).

The acoustic characteristics of these calls are quite different from the rat pup isolation calls. 22 kHz calls have a relatively stable frequency (20-30 kHz) with minimal modulation (1-6 kHz bandwidth; Sales, & Pye, 1974; Brudzynski et al., 1993; Brudzynski & Pniak, 2002; Brudzynski, 2005; 2009; Portfors, 2007). There is a short version of this call (lasting 20-300 ms) and a long version (300-3400 ms; Sales, & Pye, 1974; Brudzynski et al., 1993; Brudzynski & Pniak, 2002; Brudzynski, 2005; 2009). The call duration of this type of call is clearly quite variable and it is speculated that this parameter, along with the total number emitted, is where the information coding lies (Brudzynski et al., 1993; Brudzynski & Pniak, 2002; Brudzynski, 2005; 2009).

These alarm calls are generated by release of acetylcholine in the medial cholinoceptive strip in the brain that originates from the laterodorsal tegmental nucleus and innervates the periventricular areas, anterior hypothalamic preoptic area, bed nucleus of the stria terminalis, and the lateral septal nucleus (Brudzynski, 1994; Brudzynski & Barnabi, 1996; Brudzynski, 2001; 2007; 2009). The endogenous release of acetylcholine in this system can be mimicked by application of muscarinic cholinergic agonists (i.e., carbachol) into the post-synaptic fields, while anticholinergic agents (i.e., atropine or scopolamine) applied to the same areas decrease the amount of 22 kHz calls emitted (Brudzynski, 1994; 2001; 2005; 2009).

The 22 kHz vocalizations serve a communicative purpose because they are capable of eliciting behavioural change in conspecifics. For example, when natural and even simulated 22 kHz calls were played back to rats, these calls elicited freezing, avoidance behaviours, or bursts of locomotion; all consistent with defensive behaviours (Brudzynski, et al., 1993; Brudzynski &

Chiu, 1995; Wöhr & Schwarting, 2007; Portfors, 2007). Similarly, Blanchard et al., (1991) demonstrated that when alarm calls were emitted by a dominant rat in response to a predator in an open area outside of a colony burrow, the entire colony responded by hiding, demonstrating defensive behaviours, and propagating the message by further emitting 22 kHz calls for hours.

The 22 kHz calls also have relevance in other social contexts. During inter-male aggression, these calls were found to be associated with submissive postures and the occurrence of the dominant attacks decreased as the submissive 22 kHz calls increased (Sales, & Pye, 1974; Lehman & Adams, 1977; Sales, 1979a; Portfors, 2007; Burgdorf, et al., 2008). Also, individually reared rats with a subsequent lack of social interaction tend to emit fewer of the 22 kHz calls in response to aversive stimuli than rats reared in paired housing with a same sex conspecific (Inagaki, et al., 2005).

50 kHz Vocalizations

Mutually exclusive to, and opposite in biological function to the 22 kHz alarm call is the 50 kHz appetitive ultrasonic vocalization. This separation in signal meaning by frequency range ensures that the call receiver recognizes the message within the signal and can then respond accordingly (Brudzynski, 2007). This type of call is also emitted in adulthood and in adolescence and is observed during rough-and-tumble play (Panksepp, 1981; Knutson, et al., 1998; Portfors, 2007; Burgdorf, et al., 2008), in anticipation of social contact (Knutson et al., 1998; Brudzynski & Pniak, 2002; Wöhr, et al., 2008; Brudzynski, 2009), handling (Brudzynski & Ociepa,1992), mating (Barfield & Geyer, 1972; Sales & Pye, 1974; Sales, 1979a; White & Barfield, 1987; 1989; 1990; Kaltwasser, 1990; Portfors, 2007), in response to an anaesthetized conspecific (Blanchard, et al., 2003), rewarding brain stimulation (Burgdorf et al., 2000), or in response to an

addictive drug (Brudzynski, 2009). Rats also emit such calls when "tickled" by a skilled experimenter in a playful way resembling rat rough-and-tumble play (Knutson et al., 1998; Panksepp et al., 2000; Burgdorf & Panksepp, 2001; Portfors, 2007). In general, 50 kHz vocalizations are emitted during appetitive situations and are a postulated index of the rat's positive affective state (Burgdorf et al., 2000; Knutson et al., 2002).

This type of adult vocalization is initiated by dopaminergic stimulation of the mesolimbic pathway (Ikemoto & Panksepp, 1999; Burgdorf et al., 2007; Burgdorf et al., 2008; Brudzynski, 2009). This pathway originates in the ventral tegmental area (VTA) and innervates the nucleus accumbens shell and other forebrain structures (Burgdorf et al., 2001; Thompson et al., 2006; Brudzynski, 2007). Dopaminergic agonists such as amphetamine and apomorphine injected directly into the nucleus accumbens shell mimick the endogenous dopamine release from the VTA and elicit 50 kHz vocalizations (Burgdorf et al., 2001; Thompson et al., 2006; Brudzynski, 2007). Likewise, intraaccumbens injection of dopaminergic antagonists attenuate the 50 kHz calling response (Thompson, et al., 2006; Burgdorf, et al., 2007; Brudzynski, 2007).

These calls are characterized by a short duration of 3-65 ms, a frequency range of 35-75 kHz, and a variable bandwidth due to variation in frequency (Sales, 1972a; Sales, & Pye, 1974; Blanchard et al., 1991; Brudzynski & Pniak, 2002; Brudzynski, 2007; 2009). Due to many variable acoustic parameters, it is possible to code the information in the signal in a variety of ways (Portfors, 2007; Wöhr, et al., 2008); however, it has been suggested that the meaning of this type of call is encoded in the number of emitted calls per unit time and/or in the frequency modulation (Brudzynski, 2005). In previous studies, it was found that in positive social

situations, rats emitted significantly more 50 kHz calls than in socially irrelevant ones; further suggesting that the message is likely encoded in the number of calls emitted per unit time (Brudzynski, 2005).

Two Types of 50 kHz Vocalizations

Recently, researchers have been able to distinguish two major types of 50 kHz calls: constant frequency (flat) calls and frequency-modulated calls (step calls with trills; Burgdorf et al., 2007, Wöhr, et al., 2008; Burgdorf et al., 2008). In the past, all types of 50 kHz calls have been labelled as the appetitive or positive ("happy") rat calls. However, more recent research has been showing that there is a shorter, constant frequency call (flat call) that was interpreted as having a social co-ordinating function during inter-male aggression or as a means of expression of social ambivalence (Wöhr, et al., 2008; Burgdorf, et al., 2008). In resident-intruder paradigms, the intruder was the source of the flat 50 kHz calls suggesting that the rat was showing its submissiveness when entering the resident's home cage in an attempt to minimize attacks (Wöhr, et al., 2008). Perhaps the calls were emitted as if to say, "approaching in a friendly manner" to the rat in their home cage (Brudzynski, & Pniak, 2002). There has been evidence of some approach behaviour elicited from playback of flat 50 kHz calls, yet the rats more readily approached the source of the frequency modulated 50 kHz calls (Wöhr, & Schwarting, 2007).

Although the flat 50 kHz call may serve a purpose other than an unambivalent positive affective state, the 50 kHz frequency modulated calls do consistently correlate with an appetitive state in the rat (Burgdorf, et al., 2008; Brudzynski, 2009). For example, rats would choose playback of the frequency modulated 50 kHz call over the flat variety at significance levels

(Burgdorf, et al., 2008). Also, it has been demonstrated that these calls induce approach behaviour from rats receiving playback of 50 kHz calls from both sexual, and non-sexual situations, i.e., rough-and-tumble play (Wöhr & Schwarting, 2007; Burgdorf, et al., 2007; 2008). Even juvenile rats would prefer to be near adult rats that emitted high levels of frequency modulated 50 kHz vocalizations and would avoid those emitting 22 kHz calls (Panksepp et al., 2002; reviewed in Burgdorf et al., 2008). Previous experiments demonstrating that 50 kHz calls indicate a positive social experience, (i.e., in anticipation of social contact and play), are still correct in their interpretation of 50 kHz calls as being indicative of a positive state in the animal; however, newer research indicates that frequency modulated 50 kHz calls are more effective in displaying the positive affective state than the flat 50 kHz call (Knutson et al., 1998; Bialy et al., 2000; Brudzynski & Pniak, 2002; Burgdorf, et al., 2007).

Panksepp & Burgdorf (2000; 2003) have proposed that the frequency modulated 50 kHz calls may represent an archaic form of human laughter. These calls are seen during heterospecific and conspecific play and are emitted during times of positive affect much like when humans laugh (Panksepp & Burgdorf, 2000; 2003; Burgdorf, et al., 2008). However, this does not mean that the calls the rats emit are exactly laughter, but rather that they are a counterpart of human laughter (Bruzynski, 2009). Research into the exact meaning and behavioural consequences of the various types of frequency modulated 50 kHz calls is the direction in which much rat vocalization studies are going.

Since 50 kHz calls have been seen to reliably and effectively exhibit positive emotional states in the rat, they have received much experimental attention (Wöhr & Schwarting, 2007), and could possibly be of use in studying emotional disorders as potential models of emotional

disorders in humans. Also, because it has been well documented, as mentioned previously, that 50 kHz calls are emitted by activity of the mesolimbic pathway, these calls may serve as a unique measure in studying natural reward pathways (Wöhr & Schwarting, 2007; Wöhr, et al., 2008). Thus, many studies are focussing on the functional role of dopamine receptor subtypes in 50 kHz vocalization behaviour.

Dopaminergic Receptors – D_2 and D_3 Subtypes

Dopamine (DA) is a monoamine neurotransmitter in the central nervous system (CNS; Gehlert et al., 1992; Neve & Neve, 1997; Kebabian et al., 1997; Webster, 2001; Cooper, et al., 2003; Julien, 2005). In the CNS, dopamine is involved in motor regulation, reinforcement, olfaction, mood, concentration, hormone control, and hypoxic drive (Julien, 2005). The overall concentration of DA in different brain regions is as follows: the striatum (10 μg/g), nucleus accumbens (5 μg/g), olfactory tubercle (6 μg/g), and the cortex (0.1 μg/g) (Webster, 2001).

Through various cloning studies, five different varieties (or subtypes) of dopamine receptors have been discovered (Kruk & Pycock, 1991; Gehlert et al., 1992; Neve & Neve, 1997; Kebabian et al., 1997; Webster, 2001; Von Bohlen, et al., 2002; Cooper et al., 2003; Julien, 2005). There are two families of receptors with various subtypes. The D1 receptor family includes the D_1 and D_5 receptors. The D2 receptor family includes the D_2, D_3, and D_4 receptors. Each family is characterized by different pharmacological and biochemical properties although subtypes within the families are somewhat related in function (Kruk & Pycock, 1991; Gehlert et al., 1992; Neve & Neve, 1997; Kebabian et al., 1997; Webster, 2001; Von Bohlen, et al., 2002; Cooper et al., 2003; Julien, 2005). All types of dopaminergic receptors have seven membrane

spanning regions and are metabotropic - have interactions with G-proteins to exert their effects (Neve & Neve, 1997; Kebabian et al., 1997; Von Bohlen, et al., 2002; Cooper, et al., 2003; Julien, 2005).

The D_2 receptor subtype relative abundance in the brain (in decreasing concentration) is as follows: striatum, limbic regions, spinal cord, hypothalamus, and hippocampus (Webster, 2001). There are long and short isoforms of this receptor although both seem to exert the same effects when the receptor is activated (Gehlert et al., 1992; Neve & Neve, 1997; Kebabian et al., 1997; Webster, 2001; Von Bohlen, et al., 2002). D_1 and D_2 receptors have been implicated in the stimulatory effects seen within the nigrostriatal pathway and also in the mesolimbic pathway in eliciting 50 kHz vocalizations. D_2 receptors have been studied more so than the D_3 subtypes and this may be due to the more abundant variety of antagonists and agonists available for studying the D_2 receptor.

The D_3 receptor has structural and pharmacological similarities with the D_2 receptor because they are in the same family of dopamine receptors. Both subtypes inhibit adenylate cyclase; however, the exact G-proteins involved for each subtype may vary (Neve & Neve, 1997; Kebabian et al., 1997; Webster, 2001). D_3 receptors are distributed in the basal forebrain, the olfactory tubercule, nucleus accumbens, and islands of Calleja, (Sokoloff et al., 1990; Gehlert, et al., 1992; Neve & Neve 1997; Webster, 2001; Von Bohlen, et al., 2002); however, they are found mainly in the mesolimbic pathway which is associated with motivational and emotional functions (Sokoloff et al., 1990; Gehlert et al., 1992; Neve & Neve, 1997; Cooper, et al., 2003).

Due to a lack of selective specificity of D_3 agonists and their antagonists, there is nothing known about its role in the production of 50 kHz vocalizations.

There are post-synaptic and pre-synaptic forms (autoreceptors) seen for both D_2 and D_3 DA receptors, and there has been an abundance of mRNA found in the rat brain for the D_2 and D_3 varieties of dopaminergic post-synaptic receptors in the nucleus accumbens (Sokoloff et al., 1990; Gehret et al., 1992; Elsworth & Roth, 1997; Webster, 2001; Cooper, et al., 2003). By activating post-synaptic receptors, there is an enhancement in DA transmission while blocking post-synaptic receptors subsequently attenuates DA transmission. By activating DA autoreceptors, a negative feedback loop is initiated that will decrease synthesis and release and possibly decrease the firing rate of the pre-synaptic neuron. Likewise, if a DA autoreceptor is blocked, DA synthesis and release is increased (Elsworth & Roth, 1997; Von Bohlen, et al., 2002; Cooper, et al., 2003). The autoreceptors, like postsynaptic receptors, desensitize in response to DA agonists and become supersensitive after repeated DA antagonist application (Elsworth & Roth, 1997; Von Bohlen, et al., 2002; Cooper et al., 2003). However, if there are minimal amounts of DA in the synapse or the frequency of release is too low, autoreceptors are not activated; suggesting there is an autoreceptor threshold for activation (Webster, 2001). It seems that these DA autoreceptors are most common in striatal and mesolimbic circuits and are most often of the D_2 and D_3 receptor subtype (Elsworth & Roth, 1997; Webster, 2001).

Mesolimbic Dopamine System

Dopamine has three main pathways in the brain: mesocortical/mesolimbic, nigrostriatal, and tuberoinfundibular (Kruk & Pycock, 1991; Webster, 2001; Cooper, et al., 2003; Julien,

2005). The mesolimbic pathway is involved in reward reinforcement, and emotion; the nigrostriatal pathway is heavily involved in motor regulation and control; the tuberoinfundibular pathway is involved in the regulation of hormone release. The pathway focussed on for this thesis is the mesolimbic pathway as it is involved in emotional behaviour (50 kHz calls are an index of positive affective states), and it is capable of stimulating the 50 kHz call response after direct DA agonist application. The dopamine neurons originate from the ventral tegmental area (VTA) and innervate mostly the nucleus accumbens shell. The mesocortical pathway is connected by further projections to forebrain areas (Kruk & Pycock, 1991; Neve & Neve, 1997; Webster, 2001; Cooper, et al., 2003; Julien, 2005). An enhancement in dopamine transmission in the mesolimbic system is linked with reinforcing effects of psychostimulant drugs like amphetamine and apomorphine (Ranaldi & Beninger, 1994; Von Bohlen, et al., 2002; Cooper, et al., 2003). The 50 kHz vocalizations, previously discussed, are emitted following dopamine release in the nucleus accumbens shell as part of the mesolimbic dopamine system (Ikemoto & Panksepp, 1999; Burgdorf et al., 2007; Burgdorf et al., 2008; Brudzynski, 2009). Also noteworthy is that the mesoaccumbal dopaminergic system responds to DA antagonists, DA agonists, and responds to monoamine oxidase (MAO) inhibitors. The DA nerve terminals contain synthesis modulating autoreceptors, and have high-affinity DA transporters (Gehlert et al., 1992; Neve & Neve, 1997; Cooper, et al., 2003). The ability to respond to the aforementioned types of pharmacological agents suggests that manipulation of the system and resulting mesolimbic response is possible through drug application to the mesolimbic dopamine system.

It is a basic principle of pharmacology that the pharmacological, physiological, or behavioural effects induced by a drug follow from the interaction of ligands with receptors (Neve & Neve, 1997; Julien, 2005). It is also a general rule of psychopharmacology that drugs do not create any unique artificial effects; they merely modulate normal neuronal functioning - mimicking, or antagonizing the actions of a specific neurotransmitter, i.e., dopamine (Julien, 2005). Binding accompanied by drug-induced mimicry or facilitation of neurotransmitter action is an agonistic action. Drug occupation of a receptor that is not accompanied by neurotransmitter-like activation and blocks the access of the neurotransmitter to the receptor is an antagonistic action (Kruk & Pycock, 1991; Neve & Neve, 1997; Julien, 2005).

Quinpirole

The drug chosen to be used as a means of manipulating the mesolimbic system (mimicking DA release in the nucleus accumbens shell from VTA activation), with respect to 50 kHz vocalization behaviour is quinpirole. Quinpirole is a dopaminergic D_2/D_3 receptor agonist (Koller et al., 1987; Mogenson & Wu, 1991a; 1991b; Szechtman et al., 1994; Neve & Neve, 1997). It has been reported as having a higher affinity for the D_2 receptor than the D_3 receptor (Kruk & Pycock, 1991; Kebabian et al., 1997). However, some suggest that it has a higher affinity for the D_3 receptor than the D_2 receptor (Gehlert et al., 1992; Neve & Neve, 1997; Cooper, et al., 2003). Nonetheless, most research reported using this drug, has been with respect to locomotion in rats whether it be via systemic injection or intracerebral injection.

Repeated treatment with quinpirole produces a sensitized behavioural response in rats manifested as an increase in locomotor activity (Szechtman, et al., 1994; Neve & Neve, 1997;

Lomanowska, et al. 2004; Culver et al., 2008). This sensitization is a common response with most dopaminergic psychostimulants that seems to be caused by heightened mesolimbic system activation (Wu et al., 1993; Szechtman et al., 1994; Culver, et al., 2008). Particular interest has been placed in the quinpirole sensitized rat. Through repeated intermittent administration of quinpirole, rats become sensitized and engage in compulsive "checking" behaviours akin to obsessive compulsive disorder (OCD) in humans; quinpirole sensitization has been suggested as a possible animal model for OCD (Culver, et al., 2008).

Quinpirole also elicits changes in rat locomotor behaviour after acute administration. These effects are generally biphasic resulting in the initial inhibition of behaviour followed by excitiation (Szechtman et al., 1994; Culver, et al., 2008). At lower doses (0.1 mg/kg), quinpirole produces locomotor inhibition (Koller et al., 1987; Mogenson & Wu, 1991a), whereas higher doses display this biphasic effect (Lomanowska, et al., 2004; Culver, et al., 2008). As the number of injections increases (sensitization), the latency of hyperkinesia onset decreases (Sullivan, et al., 1998; Lomanowska, et al., 2004). It has been suggested that this inhibition effect seen after acute quinpirole application is likely due to autoreceptor activation (Mogenson & Wu, 1991a; 1991b; Wu et al., 1993; Neve & Neve, 1997; Lomanowska, et al., 2004); there is much evidence in general of the dopamine autoreceptor activation by quinpirole (Koller, et al., 1987; Mogenson & Wu, 1991a; 1991b; Wu et al., 1993).

After acute administration of quinpirole, the treatment increased dopamine tissue levels in the nucleus accumbens and in the right prefrontal cortex (Sullivan et al. 1998). Sullivan, et al., (1998) reported that in a post mortem study completed by Chen, et al., (1987), it was found that

15 min after a 1 mg/kg injection of quinpirole, dopamine stores were increased by 39% in the nucleus accumbens, 23% in the caudate, and 19% in the frontal cortex. There were also decreases in accumbens and caudate DOPAC levels suggesting a decreased turnover of dopamine, which could be the cause of the increased locomotor behaviour elicited after the initial decrease in activity. Also, after sensitization treatment, quinpirole increased the density of D_2 dopamine receptors in the nucleus accumbens, but not in the striatum (Culver, et al., 2008). This change in receptor density could correlate with an increased sensitivity of dopamine autoreceptors (Lomanowska, et al., 2004; Culver, et al., 2008). It is therefore evident that quinpirole has an effect on the dopaminergic system pre- and post-synaptically; however, the exact mechanism of action is unknown at this time.

Amphetamine

Amphetamine is a well-known and widely used psychostimulant that increases the release of dopamine from pre-synaptic nerve terminals enabling the released DA to act on post-synaptic terminals (Mogenson & Wu, 1991b; Wu et al., 1993; Webster, 2001; Von Bohlen et al., 2002; Cooper et al., 2003; Julien, 2005; Fleckenstein et al., 2007). Through action in the CNS, amphetamine produces behavioural effects of tremor, restlessness, increased motor activity, agitation, insomnia, and a loss of appetite (Julien, 2005). Amphetamine-increased dopaminergic activity at postsynaptic terminals results in increased locomotor behaviour and sometimes stereotyped behaviour when given in high doses (Mogenson & Wu, 1991b; Szechtman et al., 1994; Julien, 2005). At lower doses, amphetamine elevates arousal and causes an overall increase in positive mood and speech behaviour (Julien, 2005). Amphetamine also increases 50 kHz calling rates when directly injected into the nucleus accumbens shell of rats (Burgdorf et al.,

2001; Thompson et al., 2006; Brudzynski, 2007; Ahrens et al., 2009). Particularly, the numbers of frequency modulated call types are elevated (Burgdorf et al., 2001; Ahrens et al., 2009).

Amphetamine, similarly to cocaine, is taken up by the pre-synaptic dopamine transporter (DAT) into pre-synaptic nerve terminals where it causes the release of dopamine from vesicles (Kruk & Pycock, 1991; Webster, 2001; Julien, 2005; Fleckenstein, et al., 2007). Amphetamine is a MAO inhibitor and thus stops the breakdown of DA released from vesicles. DA then travels down a concentration gradient out of the pre-synaptic cell into the synaptic cleft. Alternatively, amphetamine may enter the pre-synaptic cell by means of exchange diffusion with DA (Kruk & Pycock, 1991; Webster, 2001; Julien, 2005). In the present study, amphetamine is used as the positive control for quinpirole dopaminergic stimulation as it is well documented that amphetamine elicits 50 kHz vocalizations from the accumbens even though the molecular mechanism of action of the two drugs may differ.

Rationale and Hypotheses

Previous research completed in the lab here at Brock University (St. Pierre, 2008) suggested that intraaccumbens injection of quinpirole significantly increased the number of 50 kHz calls beyond those elicited by isotonic saline. However, this result was seen in the high dose range (from 0.5 – 20 μg in 0.2 μL), and the dose response had not been fully completed with respect to the low dose range. The goal of this thesis was to explore the low dose range (0.025 – 1.0 μg in 0.2 μL), and determine if intraaccumbens injection of quinpirole in low doses would also significantly increase 50 kHz vocalizations. It may also be expected that quinpirole may have an atypical dose-response curve. Also, as previously mentioned with regard to locomotor studies, quinpirole's acute injection effects are biphasic (initial decrease in activity followed by

an increase in activity; Szechtman, et al., 1994; Culver, et al., 2008). It was of interest to determine if there would be biphasic effects with vocalization behaviour after direct application of quinpirole to the accumbens shell.

The findings in the higher dose range (St. Pierre, 2008) were consistent with literature stating that dopaminergic agonists injected directly to the nucleus accumbens shell elicit 50 kHz vocalizations (Webster, 2001; Burgdorf, et al., 2001; Thompson et al., 2006; Ahrens, et al., 2009). To my knowledge, this study is the first research done with quinpirole and its effects on 50 kHz calls in rats in the low range of doses. Thus, it was imperative to complete, in detail, the dose response to be able to explain quinpirole's overall effects on 50 kHz calls.

Along with looking at the possible increase of 50 kHz calls after low dose quinpirole application, it was of interest to explore the sonographic properties of the calls emitted as well. As aforementioned, much of the ultrasonic vocalization field is working toward the behavioural meaning and consequence of the different subtypes of 50 kHz calls. Was quinpirole increasing all types of 50 kHz calls proportionally or was there a specific type of call being increased, particularly frequency modulated calls as happens after amphetamine administration.

The specific hypotheses constructed were:

1) It was expected that the low dose range of quinpirole would not significantly increase 50 kHz calls beyond saline levels. Work completed by St.Pierre (2008) showed the 0.5 μg dose of quinpirole to be ineffective in stimulating the 50 kHz call behaviour. There was no evidence to suggest that the low dose range could stimulate behavioural activation.

2) The standard call parameters and sonographic properties of the calls were not hypothesized to be affected by quinpirole.

3) Raclopride antagonism at the higher dose level of quinpirole was successful (St. Pierre, 2008). Therefore, raclopride (D_2 receptor) antagonism was also planned and a successful attenuation of any quinpirole effect, if seen, was expected.

4) Since quinpirole also has affinity for the D_3 subtype of dopamine receptor, a D_3 antagonist (U99194A – maleate) was planned to be used for comparison with the D_2 antagonist (raclopride), but not expected to antagonize the response because of previous work completed by St. Pierre (2008) where raclopride successfully antagonized the 50 kHz call response at high doses of quinpirole.

5) If any increase in calls was observed after quinpirole application, it was hypothesized that the frequency modulated variety of calls would be increased as well, as is observed after application of amphetamine.

Significance of the Study

As previously reported, 50 kHz calls are elicited after dopaminergic agonist application in the mesolimbic pathway. This pathway has long been implicated in drug reward, natural reward, emotionality, and motivation (Kruk & Pycock, 1991; Neve & Neve, 1997; Webster, 2001; Cooper, et al., 2003; Julien, 2005). Also, 50 kHz vocalizations (especially the frequency modulated type) have been found to be an index of positive affect in the rat and are elicited through stimulation of the mesolimbic pathway (Burgdorf et al., 2001; Thompson et al., 2006; Brudzynski, 2007). Perhaps 50 kHz vocalizations can help elucidate some aspect of human

emotionality and possibly addiction. Human emotional and motivational disorders such as depression and anxiety are steadily rising to the second cause of financial burden to the health care system in North America (World Health Organization, 2010). If a suitable animal model of human emotional disorder(s) is possible through measurements of ultrasonic vocalizations, it is of much importance to explore and hopefully discover ways in which these calls are elicited, produced, and what receptors are specifically responsible so that drugs with an affinity to these receptors can be developed and tested.

Aside from emotional human disorders, human motor disorders such as Parkinson's disease (PD) have been utilizing the method of USV analysis to help study the communicative symptoms in rat models of PD (Ciucci, et al., 2007; Ciucci, et al., 2010). The USV deficits observed in the rat models, (i.e., decreased volume, and degradation of pitch/tone, and segmentation of calls), are also those observed for humans suffering from this debilitating disorder (Ciucci, et al., 2007; Ciucci, et al., 2010). Through the study of the vocal aspect of the disease, possible treatments may come available. USVs are a valid and extremely useful tool when looking to study and possibly assuage some of the symptoms of human disease whether it be emotional or vocal communication disorders.

Materials and Methods

Experimental Subjects

The subjects used throughout this thesis were 85 adult male Wistar rats weighing approximately 220-230g upon arrival to Brock University from Charles River (Montreal, QC, Canada). Rats were pair housed in standard polycarbonate cages (dimensions: 460 mm x 250 mm x 195 mm) with corn cob bedding, one black polyvinyl tube for hiding, and two sheets of paper towel. Rats were given approximately one week to acclimate to the facility. The cages underwent a thrice weekly cleaning schedule and received standard rat chow and water available ad libitum, on a 12/12hr light/dark cycle. All experimental procedures were done in accordance with the guidelines set by the Canadian Council on Animal Care and were approved by the Animal Care and Use Committee at Brock University.

Stereotaxic Surgeries

At the time of surgery, all rats weighed approximately 250-340g. Animals were anesthetized by an intraperitoneal injection of ketamine hydrochloride (40 mg/kg i.p., Katalean, MTC Pharmaceuticals, Cambridge , Ont. Canada) and xylazine hydrochloride (6 mg/kg i.p., Rompun, Bayvet Div. Chemargo Ltd., Etobicoke, ON, CA) and were placed in the Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) after shaving the surgical site. Once properly mounted to the apparatus, the surgical site was scrubbed with an iodine and detergent solution. This was followed by a cleaning with isopropyl alcohol and finally, a pure iodine solution was administered to the site. A small incision was made in the scalp, and the periosteum was scraped away revealing the skull bone sutures. The apparatus was then calibrated and set to the appropriate co-ordinates for the nucleus accumbens shell according to the Paxinos

& Watson, (1986) stereotaxic atlas. Small holes were drilled in the skull and chronic 23 gauge stainless steel guide cannulae were implanted bilaterally to the nucleus accumbens shell. The co-ordinates used for the nucleus accumbens shell measured from lambda were A = 9.7 mm, L = 1.2 mm, V = 6.7 mm and were placed 1 mm above the intended injection site (Paxinos, & Watson, 1986). Following cannulae implantation, stainless steel jeweller's screws were used along with methyl methylacrylate resin (Perm, Hygenic Corporation of Canada Inc., St. Catharines, ON, CA) to secure the implant(s). Finally, the incision was sutured around the acrylic resin. After surgery, the openings of the cannulae were plugged with stainless steel wires and rats received a subcutaneous injection (s.c.) of an analgesic, ketoprofen (2 mg/kg s.c., Anafen, Merial Canada Inc., Morgan Baie d'Urfe, QC, CA) and an antibiotic, enrofloxacin (11 mg/kg s.c., Baytril, Bayer Inc., Toronto, ON, CA), and were allowed to recover for 7 days in isolation housing conditions. For more information on the stereotaxic cannulae implantation surgeries, see Pellegrino & Cushman (1971), & Myers, (1971).

Intracerebral (Intraaccumbens) Single Injections and Drugs

Groups of rats underwent surgery each within a few days to minimize the differences in the time of survival and possibility of infection. As a result, injections were organized and carried out in approximately groups of fifteen rats at a time. The first three groups of rats underwent the dose response injections (quantifying the drug-receptor interaction; Julien, 2005), which were done unilaterally with dosages of 0.025 μg, 0.06 μg, 0.12 μg, 0.25 μg, 0.5 μg, and 1.0 μg quinpirole hydrochloride (Sigma-Aldrich Canada Ltd., Oakville, ON, CA) and isotonic saline (vehicle control) injected through the right guide cannula (see Appendix A for injection schedule). A 7 μg dose of D-amphetamine sulfate (Dextroamphetamine, Sigma-Aldrich Canada Ltd., Oakville, ON, CA) was injected unilaterally through the left guide cannula as a positive

control. Results of injections were recorded immediately after intracerebral drug injection. Dose response groups also underwent an additional recording 35-40 minutes after the initial injection without any additional injection. The rats spent the time lapse between recordings in their home cage inside the colony room. This was completed to determine if the effects of the drug were immediate, long-lasting, or delayed.

All injections were completed with a Hamilton constant rate micro-syringe (CR-700-20) at a rate of 3.3×10^{-6} mL/s via polyethylene tubing connected to a stainless steel 30 gauge injection cannula for a total injection volume of 0.2 μL per injection. For all groups, injection sessions were arranged with a three to four day lapse between injections. This was done to minimize any possible drug interaction or sensitization, and to allow the receptors to recover.

Intracerebral (Intraaccumbens) Double Injections and Drugs

Similarly, an additional three groups of approximately fifteen rats were used to complete the double injection antagonist portion of this thesis (see Appendix A for injection schedules). The first of the three groups received unilateral injections of D-amphetamine sulfate (Dextroamphetamine, Sigma-Aldrich Canada Ltd., Oakville, ON, CA) pre-treated with either isotonic saline or an equimolar dose of raclopride L-tartrate (Sigma-RBI, Oakville, ON, CA), a dopamine D_2 receptor antagonist. Raclopride has a K_d value of ~1.7 nM to the D_2 receptor and a K_d value of ~ 2.3 nM for the D_3 receptor (Seeman, et al., 2006). These injections were completed using the right guide cannula to avoid the sites into which the amphetamine was injected on the left side of the brain in the dose response injections. On the left side of the brain, the quinpirole hydrochloride (Sigma-Aldrich Canada Ltd., Oakville, ON, CA) injections were administered at a dose of 0.25 μg as this was found to be the optimal dose from the low dose response data

obtained in this study. These injections were pre-treated with isotonic saline or raclopride L-tartrate (Sigma-RBI, Oakville, ON, CA) in an equimolar dose to the 0.25 μg dose of quinpirole.

The final two double injection groups were injected in an identical manner with full counterbalancing across cannulae and drug condition. These rats received injections of 0.25μg quinpirole hydrochloride (Sigma-Aldrich Canada Ltd., Oakville, ON, CA) pre-treated with either isotonic saline, or an equimolar dose of U-99194A maleate (Sigma-Aldrich Canada Ltd., Oakville, ON, CA), a dopamine D_3 receptor antagonist. U99194A maleate has a K_d value of ~ 2281 nM for the D_2 receptor and a K_d value of ~223 nM to the D_3 receptor (Seeman, et al., 2006).

Vocalization Recording

Vocalization recording for all groups commenced approximately one minute after removal of the injection cannula as 50 kHz calls started to be emitted at that time. All recordings took place in a clean polycarbonate cage (dimensions: 220 mm x 200 mm x 190 mm) with fresh corn cob bedding and a wire grate lid. The recording condenser microphone (model CM16/CMPA, Avisoft Bioacoustics, Berlin, Germany) was mounted to this lid approximately 200 mm away from the rat, and was aimed directly into the cage. This method of vocalization recording is more reliable and accurate in preserving all aspects of the call and maintaining the call in its undivided form compared to past techniques using a bat detector, which utilized frequency division (Burgdorf et al., 2008; Brudzynski, 2009). After each recording session, the recording cage was replaced. This was done to minimize scent cues from other rats as these have been demonstrated to elicit 50 kHz calling in anticipation of social contact (Brudzynski & Pniak, 2002; Wöhr et al., 2008). All recordings were taken for a total time of 10 minutes per injection at a 500,000 Hz sampling rate in 16 bit format using Avisoft Bioacoustic Software (Avisoft

Recorder NI-DAQMX, Berlin, Germany) and stored on the hard drive of a PC computer (Dell Precision 390); the files were permanently stored on DVDs for re-analysis. Dose response groups underwent an additional recording 35-40 minutes after the initial injection. The time lapse between recordings was spent in their home cage in the colony room. This delay was to determine if the effects of quinpirole were immediate, long-lasting, or delayed.

Histological Localization of Injection Sites

Following injection(s) completion, rats were euthanized with an overdose of Euthanyl (240 mg/mL sodium pentobarbital, Euthanyl, Vetoquinol N-A, Lavaltrie, QC, CA), an approximate 0.5-0.8 mL intraperitoneal injection. When euthanasia was complete, brains were transcardially perfused with 10% formalin solution to fix the brain before extraction (approximately 50-60 mL of 10% formalin per rat). Brains were manually extracted and individually placed in 10% formalin solution for a minimum of 2 days before sectioning.

Fixed brains were removed from formalin, rinsed with water, and blocked to isolate the cannulae tracts. Brains were mounted to the stage of a freezing microtome (Cryo-Histomat, Hacker Instruments and Industries, Fairfield, NJ) with Tissue Tek compound (Miles Laboratories Inc., Naperville, IL, USA), and the dorsal surface was angled toward the blade. Brains were sectioned to a thickness of 40-50μm. After sectioning, brains were mounted on labelled polylysinated microscope slides by floating the slices onto the slide in a distilled water bath. They were then allowed to dry for 12-24 hours before staining.

The prepared sections were stained using a modified Nissl staining technique according to the Rucker-Koithan procedure with a thionine stain (Windle, et al., 1943; Skinner, 1971). The Nissl bodies, nucleoli, necrotic tissue, and intracerebral injection sites stained dark blue while the

fibre tracts stained a lighter blue. After the staining procedure was complete, slides were coverslipped using Permount glue (Fisher Scientific, Suwanee, GA, USA). Once given time to dry (approximately 24 hours), the slides were used to localize the cannulae tracts and injection sites. The middle point of the injection site and not the base of the guide cannulae tracts were the target for localization. This was done using a projection light microscope and the coronal sections from the rat brain atlas by Paxinos & Watson (1986).

Acoustic Analysis of 50 kHz Vocalizations

Rats were eliminated from data analysis if their control saline levels were 5 times higher or more than the average baseline level for the group. In these rats, there was minimal room for pharmacological effects to be observed. Also, there are some environmental factors that can influence these calls and are difficult to control for; for example, the conditioned response to the experimenter, and handling itself. Rats seen to be emitting 50 kHz calls due to approach or handling by the experimenter were also removed from analysis. Rats were also eliminated from analysis if their cannulae localization were found to be outside of the nucleus accumbens. No more than 1-2 extreme rats were removed per group before analysis, and these rats were deleted across all comparisons.

All acoustic files were analyzed in the Avisoft SASlab Pro program for the number of 50 kHz calls present within ten minutes along with the corresponding parameters: bandwidth (maximum minus minimum frequency), peak frequency, and call duration. Also observed and recorded were the sonographic variations in calls within the 50 kHz group of calls. Calls were sub-categorized into flat, step+trill, and FM other (frequency modulated other) call types (see Figure 20 for examples). Calls were considered flat if the frequency was relatively unchanging

and the bandwidth did not vary more than 10 kHz from the mean frequency (see Figure 20 A). Step calls resemble a rapid step up in frequency while trill calls resemble 2-6 sine waves fluctuations in frequency; in some cases, the step and trill call types could be seen in combination (see Figure 20 A, B & C). The step and trill calls are not as commonly observed, so they were grouped together as one subtype. Finally, FM other calls were defined as any other call varying in frequency that does not resemble a step or a trill type call (see Figure 20 D). For example, a sharp increase/decrease, sweeping type modulations, or any other type of frequency modulation. All acoustic analyses were completed by the experimenter who was not blind to experimental drug condition. However, with the call type and call parameters criterion, bias in this manner was not an issue.

Statistical Analyses

All calls incorporated into statistical analysis met the accepted standard parameters for a 50 kHz vocalization (Sales, 1972a; Blanchard et al., 1991; Brudzynski & Pniak, 2002; Brudzynski, 2005; 2007). Statistical analyses were completed using SPSS (SPSS software, SPSS Inc., Chicago, IL). The inter-individual variability (distribution in calling) in the data did not allow for standard parametric tests, thus, all statistics performed were repeated measures non-parametric Friedman ANOVAs followed by paired Wilcoxon Signed Ranks Tests.

Results

Dose Response Injections

Intraaccumbens injections of six doses of quinpirole (0.025 μg, 0.06 μg, 0.12 μg, 0.25 μg, 0.5 μg, and 1.0 μg), revealed an inverted U-shape dose response relationship with the optimal low dose of quinpirole being 0.25 μg (peak of inverted U-shape). This dose of 0.25 μg of quinpirole significantly increased the number of 50 kHz calls above saline baseline levels (χ^2 (3, 15) = 10.53, p= .015; $Z = 2.73$, $p < .006$); see Figure 1. All remaining doses of quinpirole failed to elicit significantly more 50 kHz calls than the saline controls. A similar increase in the 50 kHz call response was found after intracerebral injection of 7 μg of amphetamine ($Z = 2.84$, $p < .004$); see Figure 1. These results indicate that a low dose of quinpirole is sufficient to elicit the 50 kHz calling response after intraaccumbens injection in rats, much like the positive control of amphetamine but in a very narrow dose-range revealed by a single dose of 0.25 μg. This response was not accidental and was reproducible in all other experiments with antagonists.

Delayed Quinpirole Response

As previously mentioned, additional vocalization recordings were conducted 35-40 minutes after the scheduled injection and the first recording session. In all cases (other than saline), a trend of a decrease in calls was observed during each delayed recording when compared to the initial recording session (see Figure 3). There was a significant decrease in 50 kHz calling in delayed recording when compared to the initial recording session for 0.25 μg of quinpirole (χ^2 (7,15) = 20.75, p = .004; $Z = 2.61$, $p < .009$). Likewise, there was a significant decrease in 50 kHz calls over time for 7 μg amphetamine ($Z = 1.93$, $p < .05$). These results

indicate that the 50 kHz call response is sensitive to these dopamine agonists immediately after intraaccumbens application and does not increase over time; this effect is not delayed and it disappeared over a 40 minute time period (see Figure 3).

Raclopride (D_2 receptor) Antagonism

In an attempt to antagonize both the quinpirole and amphetamine increase in 50 kHz call responses, an interesting result was observed (see Figure 4). As expected and as documented in previous literature (Burgdorf, et al., 2001), 7 μg of amphetamine increased 50 kHz calls beyond saline levels (χ^2 (2, 10) = 5.90, p = .05; Z = 2.35, p < .05; see Figure 4). Also consistent with literature (Thompson, et al., 2006), is the antagonism of the amphetamine response by raclopride, a D_2 antagonist. Raclopride decreased 50 kHz calls more than four times when injected as a pre-treatment to 7 μg amphetamine (did not reach the significance level due to variability within data; Z = 1.79, p < .07). However, when raclopride was injected as a pre-treatment to a 0.25 μg quinpirole injection, it was unsuccessful in significantly decreasing 50 kHz calls (Z = 1.07, p < .24; see Figure 4). These results suggest that amphetamine increases 50 kHz calls through action at the D_2 dopamine receptor, whereas quinpirole is exerting its effects through another mechanism at low doses. This finding gave rise to the following section of this thesis.

U-99194A Maleate (D_3 receptor) Antagonism

Two groups of rats were used to explore the possibility that a D_3 receptor antagonist may antagonize the 0.25 μg quinpirole-induced response. The selective D_3 receptor antagonist U99194A maleate did indeed significantly decrease the 50 kHz call response elicited by 0.25 μg of quinpirole (χ^2 (3, 23) = 7.96, p = .047; Z = 2.66, p < .008; see Figure 5), suggesting involvement of D_3 dopamine receptors.

Call Parameters

For every call recorded, the parameters of call bandwidth, single call duration, and peak frequency were also analyzed. The peak frequency values were consistent with those in the literature (within 37-70 kHz; Sales, 1972a; Blanchard et al., 1991; Brudzynski & Pniak, 2002; Brudzynski, 2009). There were no significant differences in mean peak frequency among drug conditions in both dose response and double injection antagonism groups (see Figures 6, 8, & 11). Thus, the call frequency was not affected by dopaminergic agonist or by dopaminergic antagonist.

Likewise, the call duration was measured for every call in each drug condition and group. There was no difference in call duration among any of the drug conditions regardless of single or double injection (see Figures 7, 10, & 12). This call parameter was also within the usual range for a 50 kHz call type (3 - 65 ms; Sales, 1972a; Blanchard et al., 1991; Brudzynski & Pniak, 2002; Brudzynski, 2009). Similar to peak frequency, mean call duration was unaffected by dopamine agonist or antagonist.

When analyzing bandwidth values, there was an effect of drug group (see Figure 9). When comparing a double injection of isotonic saline to a pre-treatment of saline followed by an injection of 0.25 μg quinpirole, the mean call bandwidth was significantly increased ($Z = 2.52$, $p < 0.02$). When comparing pre-treatment of saline and raclopride followed by injection of amphetamine, raclopride pre-treatment significantly decreased mean call bandwidth ($Z = 2.31$, $p < 0.03$). All other groups and drug conditions failed to reach any significance values (see Figures 6 & 11), and the mean bandwidth values were again within the usual range for a 50 kHz call type (Sales, 1972a; Blanchard et al., 1991; Brudzynski & Pniak, 2002; Brudzynski, 2009). From these

results, it appears that bandwidth is increased by dopamine agonist, and decreased by dopamine antagonist.

Sonographic Structure and Sub-categorization

When recording call parameters, the sonographic structure of each call was obtained as well. The sonographic structures were sub-categorized into 3 distinct categories: flat, step + trill, and FM other (frequency modulated other). See Figure 20 for visual examples of sub-categories. In dose response groups (see Figure 13), the 0.25 μg dose of quinpirole and the 7 μg dose of amphetamine both significantly increased the number of flat (χ^2 (3, 15) = 8.21, p = .042; Z = 2.17, p < .03, and Z = 2.58, p < .01 respectively), step + trill (χ^2 (3, 15) = 15.27, p = .002; Z = 2.87, p < .004, and Z = 2.53, p < .011 respectively), and FM other (χ^2 (3, 15) = 14.65, p = .002; Z = 2.48, p < .013, and Z = 2.77, p < .006 respectively) type of calls. It is apparent that both dopaminergic agonists were capable of increasing all types of 50 kHz vocalization. This finding supports the overall increase in 50 kHz calls observed in dose response data.

The D_2 antagonist-treated group showed an increase in frequency modulated other calls (FM other) after injection of saline followed by 0.25 μg quinpirole (χ^2 (5, 10) = 20.28, p = .001; Z = 2.68, p < .008; see Figure 14). There was also an increase in FM other calls after injection of saline followed by 7 μg amphetamine (Z = 2.21, p < 0.03). Interestingly, when pre-treatment of raclopride was followed by an injection of 0.25 μg quinpirole, there was still a significant increase in FM other type calls (Z = 1.98, p < 0.05); see Figure 14. This effect was not observed when raclopride injection was followed by an injection of amphetamine (Z = 1.45, p < .15; see Figure 14). As previously described, the amphetamine induced call response was decreased with

a D_2 antagonist pre-treatment; it appears that the frequency-modulated calling response was also attenuated by D_2 antagonist pre-treatment.

Finally, the sub-categorization of calls obtained within the D_3 antagonist-treated group was analyzed (see Figure 15). As already observed, the 0.25 μg dose of quinpirole significantly increased both step + trill (χ^2 (3, 24) = 12.40, p = .006; Z = 2.29, p < .023), and FM other call type varieties (χ^2 (3, 24) = 8.18, p = .042; Z = 2.61, p < .01). Pre-treatment with the D_3 antagonist U99194A maleate attenuated both frequency modulated types of calls as there was no longer a significant increase in that call type (see Figure 15). This observation further supports the assumption that low doses of quinpirole are acting at the D_3 receptor sub-type and antagonize 50 kHz vocalizations.

Localization of Injection Sites

After all scheduled intracerebral injections were complete, rat brains were used to analyze the location of the injections. From the histological preparations, the injection sites were mapped on coronal sections from the rat brain atlas by Paxinos & Watson (1986; see Figures 16 & 17). All injection sites for both dose response and double injection groups were within the nucleus accumbens at co-ordinates of 10.7 - 9.7 mm from lambda. When mapping the 0.25 μg quinpirole response on the injection sites localized (see Figure 18), there was a trend for the nucleus accumbens shell to give the highest number of 50 kHz call responses when compared to the nucleus accumbens core, or even the border of the shell. Similarly, when mapping the 7 μg amphetamine responses to nucleus accumbens localization, the nucleus accumbens shell appears to be the most effective area in eliciting 50 kHz vocalizations (see Figure 19). It also seems that quinpirole induced more calls from the accumbens core than did the amphetamine (compare

Figure 18 and 19). Because the stereotaxic co-ordinates were chosen for the nucleus accumbens shell, there were too few localizations purely in the accumbens core. Therefore, statistics were impossible to perform to compare subdivisions of the nucleus accumbens (see Figures 18 & 19).

Discussion

Quinpirole Dose Response

The low dose of quinpirole that most effectively and significantly increased 50 kHz calls above saline levels was the 0.25 μg dose. The increase in the number of calls caused by the 0.12 μg dose approached the significance level ($Z = 1.83$, $p < .07$). It is possible that with injections of low doses of quinpirole there is a very narrow range for induction of vocalization behaviour. Nonetheless, the increase in 50 kHz calls above saline levels by the 0.25 μg dose was found in every group in which it was administered, indicating a reliable and reproducible finding.

Similarly, 7 μg of amphetamine (positive control) also significantly increased 50 kHz calls above saline levels in each group in which it was administered. It has been well established throughout the literature that amphetamine elicits these calls after intraaccumbens application (Burgdorf et al., 2001; Thompson et al., 2006; Burgdorf, et al., 2007; Ahrens et al., 2009). The dosage of 7 μg was used because it had been shown to be an optimal dose in previous studies (Thompson et al., 2006). The magnitude of the amphetamine-induced response was comparable to that after 0.25 μg of quinpirole.

The original hypothesis regarding the dose response before beginning this study was that there would be no significant increase in 50 kHz calls after low dose application of quinpirole. However, as demonstrated in the results section, this hypothesis was not supported. The 0.25 μg dose of quinpirole repeatedly demonstrated a significant increase in the 50 kHz calling response above saline levels.

Delayed Quinpirole Response

Dose response groups were also used to analyze potential delayed quinpirole effects. It was found that when rats' USVs were recorded 35 - 40 minutes after the injection of drug, the 50 kHz call response was significantly decreased in groups that initially had increased call responses (0.25 μg quinpirole and 7 μg amphetamine) when compared to saline groups. In systemic quinpirole injection studies looking at locomotor behaviour, biphasic effects in quinpirole's action were found (Szechtman et al., 1994; Culver, et al., 2008). It was of interest to determine if biphasic effects would also be observed with respect to 50 kHz calling rates after intracerebral application of quinpirole. This was not the case; effects were observed immediately after injection and were not long lasting as seen by the decrease in calling over time (35 – 40 minutes) for all sites and injection groups.

Double Injections- Dopamine D_2 and D_3 receptor Antagonism

Rats given a single saline intracerebral injection compared to those given a double intracerebral injection of saline did not differ significantly in the number of 50 kHz calls emitted ($p < .17$). This indicates that the double injection was not responsible for the increase in calling seen later from quinpirole and amphetamine. Similar to the single injections of 0.25 μg quinpirole, and 7 μg amphetamine, pre-treatment with saline followed by these agonists, again, elicited significantly more 50 kHz calls when compared to the double injection of saline. Saline injection did not change this response. When 0.25 μg of quinpirole was pre-treated with an equimolar dose of raclopride, a D_2 dopaminergic antagonist, the 50 kHz call response was not significantly affected. This indicates that quinpirole was perhaps exerting its actions using the D_3

dopamine receptor type and not the D_2 receptor type as previously hypothesized. In contrast, the 50 kHz call response induced by the 7 μg dose of amphetamine was significantly decreased by an equimolar pre-treatment with raclopride, confirming involvement of D_2 dopamine receptors (Thompson et al., 2006). The hypothesis constructed at the start of this thesis was that quinpirole's effects should be attenuated by the D_2 antagonist raclopride because this was the case with work previously accomplished in our lab (St. Pierre, 2008). This hypothesis was defeated; which led to a new hypothesis that at low doses, quinpirole must work through action at another receptor, presumably the D_3 receptor subtype.

To investigate the lack of antagonism of the quinpirole response by raclopride, two more groups of rats were used to examine the dopaminergic receptor involved in the observed vocalization response. Since quinpirole is known to be a D_2 and a D_3 dopaminergic agonist, it is likely that, if the 50 kHz call response was not effectively antagonized by means of a D_2 dopaminergic antagonist (raclopride), then it should be antagonized by a D_3 antagonist. Indeed this was the case. When rats were pre-treated with U-99194A maleate, a specific D_3 dopaminergic antagonist, there was a significant decrease in 50 kHz calls when compared to those elicited by injection of 0.25 μg of quinpirole pre-treated with saline. The D_3 dopamine receptor has not previously been implicated in the production of 50 kHz calls. There is evidence that D_1 and D_2 dopamine receptors in the nucleus accumbens shell can increase the amount of 50 kHz calling when activated (Ranaldi & Beninger, 1994; Burgdorf et al., 2001; Thompson et al., 2006; Burgdorf et al., 2007). Because the D_3 receptor subtype is also present in the mesolimbic DA system and is a member of the D2-like receptor family (Sokoloff et al., 1990; Gehret et al., 1992; Neve & Neve 1997; Webster, 2001; Von Bohlen, et al., 2002), it is plausible that this

receptor can exert similar effects when activated by quinpirole at the 0.25 μg dose. This result is the first evidence that the activation of the D_3 dopaminergic receptor type in the nucleus accumbens can elicit 50 kHz calls and confirmed the hypothesis that quinpirole may work at the D_3 dopamine receptor subtype.

Proposed Mechanism of Action for Quinpirole

The dose found to be the most effective at eliciting 50 kHz calls from the accumbens shell was the 0.25 μg dose; an effect reproduced many times in this thesis. Perhaps this dose of quinpirole was minimal enough to stimulate the D_3 post-synaptic receptors without autoreceptor activation. Autoreceptors are responsible for auto-inhibition of a neuron's transmitter release by dampening DA synthesis and release (Elsworth & Roth, 1997; Von Bohlen, et al., 2002; Cooper, et al., 2003). It has been found that both D_2 and D_3 dopamine receptors in the nucleus accumbens have post-synaptic receptors and autoreceptors on pre-synaptic neurons (Sokoloff et al., 1990; Gehret et al., 1992; Neve & Neve, 1997; Elsworth & Roth, 1997; Webster, 2001; Cooper, et al., 2003). A threshold for autoreceptor activation has been suggested (Webster, 2001), and quinpirole could act at the post-synaptic site and remain at the sub-threshold dose for the pre-synaptic autoreceptors. For dosages smaller than 0.25 μg (0.025 μg, 0.06 μg, 0.12 μg), the amount of drug could be simply not sufficient to elicit a response from the post-synaptic receptors (sub-threshold for post-synaptic response). The dosages higher than 0.25 μg quinpirole (0.5 μg & 1.0 μg), may have reached the threshold for autoreceptor activation, initiating a negative feedback loop subsequently decreasing the amount of DA release from pre-synaptic neurons available for post-synaptic activation in the synapse and subsequently decreasing the number of emitted calls. For example, in an experiment completed by Wu et al., (1993), it was

found that a 4 μg / 0.2 μL injection of quinpirole into the nucleus accumbens significantly decreased the amount of locomotor behaviour observed. It was suggested that this inhibition of activity was due to autoreceptor activation. Also, as previously mentioned in the introduction, there has been evidence of autoreceptor activation being the cause of the initial decrease in locomotor behaviour being seen following acute systemic injections of quinpirole (Koller et al., 1987; Mogenson & Wu, 1991a; 1991b; Wu et al., 1993; Neve & Neve, 1997; Lomanowska, et al., 2004).

Other work completed at Brock University (St. Pierre, 2008) suggests that once reaching higher doses of quinpirole, there are again significant increases in 50 kHz calls. At higher dosages, the synapses are most likely overwhelmed with the influx of agonist that even with autoreceptor activation, D_2 and/or D_3 post-synaptic activation is sufficient and thus gives an increase in 50 kHz calls. Also, there may be autoreceptor desensitization (Elsworth & Roth, 1997; Von Bohlen, et al., 2002; Cooper et al., 2003) resulting in even more post-synaptic stimulation and a subsequent increase in calls. It seems clear that quinpirole has action at pre-synaptic as well as post-synaptic dopamine receptor sites. This differential affinity could be the cause of the narrow dose range found in this thesis as well as the supposed bi-modal effects of quinpirole on 50 kHz vocalization behaviour.

Higher doses of quinpirole are antagonized by the D_2 antagonist raclopride as found in our lab (St. Pierre, 2008). In this thesis, low doses of quinpirole are not antagonized by raclopride but rather the D_3 antagonist U-99194A maleate. It has been suggested that quinpirole has a higher affinity for the D_3 receptor type (Sokoloff et al., 1990; Gehret et al., 1992; Neve &

Neve, 1997; Cooper et al., 2003), making it likely that low doses preferentially bind with D_3 receptors over the D_2 receptor type. The results from this thesis support the conclusion that quinpirole has a higher affinity for the D_3 receptor sub-type; however, further speculation into the mechanism of action of quinpirole is beyond the scope of the data of this thesis.

Sub-categorization of call types

There may be a shift in understanding the biological role of 50 kHz calls. Previous research suggested that all types of 50 kHz calls are associated with positive affective states in rats (Bialy et al., 2000; Burgdorf, et al., 2007; 2008; Brudzynski & Pniak, 2002; Brudzynski, 2005; 2007). More recent papers have proposed that perhaps all types of 50 kHz calls may not directly express positive affective states (Wöhr et al., 2008; Ahrens et al., 2009). However, current research is showing that flat 50 kHz calls may indicate social ambivalence or they may have a social co-ordinating function that may be associated with aggressive social contacts i.e., emitted as a means of ending an attack from a conspecific (Burgdorf et al., 2008; Wöhr et al., 2008; Ahrens et al., 2009). Whereas, the frequency modulated subtype of 50 kHz calls is where the expression of positive affect is found (Burgdorf et al., 2008; Ahrens et al., 2009). Panksepp and Burgdorf (2000; 2003) hypothesize that there is some "evolutionarily preserved correlate" between the frequency-modulated calls of rats and human laughter or joy. Both the 0.25 μg dose of quinpirole and the 7 μg dose of amphetamine not only significantly increased 50 kHz calls overall, but both dosages significantly increased the frequency modulated type of call. It is therefore plausible, based on the newly emerging hypotheses on 50 kHz vocalizations, that these drugs increase positive affect in these rats. By separating 50 kHz calls into subcategories, it can

be said that all of the previous literature is in part, correct, if we are looking at the appropriate type of 50 kHz call. In a paper by Wöhr et al., (2008) it was suggested that perhaps the separation of 50 kHz calls into distinct sub-categories may draw some human parallels. For example, in humans, there is an unfelt social smile used as a communicative gesture in social situations (perhaps akin to the flat 50 kHz call?), and the Duchene smile, one that is affectively truthful (akin to the frequency modulated variety of calls?).

The hypothesis constructed based on sonographic structure was much like that for amphetamine (Burgdorf et al., 2001; Ahrens et al., 2009). If there was a significant increase in calls produced by a low dose of quinpirole, there should be a significant increase in the frequency modulated call type. This hypothesis was confirmed by the results of the thesis. It appeared that irrespective of whether non-selective (amphetamine) or selective (quinpirole) dopaminergic agonists were used, an increase in the frequency modulated type of 50 kHz calls was observed.

Standard Call Parameters

All mean call duration and mean peak frequency values obtained in this study, did not show significant differences among groups or drug conditions. The mean values observed were within the accepted range for a typical 50 kHz call type, i.e., within 35-70 kHz mean peak frequency and 3-65 ms in duration (Sales, 1972a; Blanchard et al., 1991; Brudzynski & Pniak, 2002; Brudzynski, 2009). Interestingly, mean call bandwidth was affected by drug condition in the raclopride antagonism group. An injection of 0.25 μg quinpirole following pre-treatment with saline showed a significant increase in call bandwidth (see Figure 9). This is not a

surprising result as it is likely explained by the increase in frequency modulated vocalizations elicited by the 0.25 μg dose of quinpirole. Bandwidth is measured by taking the maximum call frequency obtained and subtracting the minimum call frequency obtained (Brudzynski, 2009). Frequency modulated calls are characterized by increased bandwidth values. When comparing the injections of amphetamine pre-treated with saline or raclopride, mean call bandwidth was significantly decreased after raclopride pre-treatment (see Figure 9). If frequency modulation is decreasing, bandwidth decreases as well; it is a positively associated relationship. This result suggests that the frequency modulated calls disappeared after antagonizing the amphetamine response with raclopride.

Localization of Intracerebral Injections

Localization of injections for both the dose response and double injection groups were concentrated between the 10.7 - 9.7 mm from lambda based on the atlas by Paxinos and Watson (1986). This localization places the majority of injections in the medial portion of the nucleus accumbens shell (see Figures 16 & 17). It has been well established that the most robust emission of 50 kHz vocalizations occurs after psychostimulant application to the medial shell (Thompson et al., 2006; Sellings et al., 2008; Ahrens et al., 2009). There have been a few studies on the anatomical localization within the accumbens relating to aversive responses and appetitive responses. The studies agree that stimulation in the rostral-medial shell results in appetitive behavioural responses, (i.e., increases in eating); whereas caudal shell stimulation results in aversion related responses, i.e., defensive treading and burying (Reynolds & Berridge, 2001; Faure et al., 2008; Sellings et al., 2008).

The nucleus accumbens has a distinct separation into the core and the shell regions (Mogenson & Wu, 1991a; Sellings et al., 2008). The core region is connected to motor circuits whereas the shell is connected to limbic circuits (Mogenson & Wu, 1991a). It has been proposed that there is an integration of information between the two sections in that emotionally or motivationally salient information is assessed in the limbic regions and is then transferred to motor or behavioural output parts through motor circuits (Mogenson & Wu, 1991a). In a paper by Sellings et al., (2008) it is explained as though the limbic regions are guiding behavioural responses based on positive or negative reinforcers. Based on this functional separation, it seems acceptable that if 50 kHz calls are an index of emotionality in rats, the calls should be elicited moreso from the shell than the core. This has been demonstrated in the past (Thompson et al., 2006), and is somewhat demonstrated in this thesis - due to a low N number for injection site localization within the core of the accumbens, proper statistical analysis could not be performed. However, when analyzing the data without statistics, it is apparent that the highest rates 50 kHz vocalizations are emitted after intraaccumbens injection in the shell region (See Figures 18 & 19).

Limitations and Variability in Data

There has been a vast inter-individual variability in call production observed repeatedly with regard to call emission (Knutson et al., 1998; Burgdorf & Panksepp, 2001; Wintink & Brudzysnki, 2001; Burgdorf & Panksepp, 2006; Schwarting et al., 2007). Some rats may have a disposition to call more. In tests conducted by Wöhr et al., (2008), it was found that there was inter-individual variability not only in call emission but also within the standard call parameters of bandwidth, peak frequency, and call duration. Schwarting et al., (2007) suggested that such

variability in individual results could be due to dispositions or traits that are characteristic to the subject under study. There have been studies in which rats were bred into three separate lines based on their propensity to call in the 50 kHz range (Burgdorf et al., 2005; Harmon et al., 2008; Burgdorf et al., 2008b). From these studies, it has been deduced that there may be a genetic predisposition to the amount of 50 kHz calls or the magnitude of positive emotion the rats express. The analysis of calls throughout this thesis supports the findings of considerable inter-individual variability in calls. This type of variability is also consistent with work completed by Wöhr et al., (2008) where it was found that when comparing Long-Evans rats with Wistar rats, there was more individual variability and call rate variability in the Wistar strain (the strain used in this thesis). This inter-individual variability is the reason for the non-parametric statistical testing used throughout this thesis as normality in the distribution was not possible.

In pharmacological studies with humans, it has been found that the dose of a drug that produces a specific response varies among patients. It is known that in any population of individuals, there will be a few that are remarkably sensitive to even low doses of drug, or in juxtaposition, are extremely tolerant to the drug (Julien, 2005). In light of this variability, many participants are required (larger N) to maximize the generalization of any drug's effects. This was a limitation in this study. Smaller group sizes of rats did not allow much room for error or extreme individual variance.

Another possible problem lies in call fragmentation; which happens when long calls are emitted at a low air pressure and the rat loses the sound (Brudzynski, 2009). The ability to recognize two sounds as either separate vocalizations or two fragments of one call comes only

with experience (Brudzynski, 2009). Throughout this thesis, the vocalization analysis was handled by one experimenter so as to eliminate any unreliability or bias across different experimenters. Since 50 kHz calls have a very short duration, fragmentation of calls is most probably not a factor in this study.

In work completed by Natusch and Schwarting (2010), it was found that environmental testing conditions are critical for 50 kHz vocalization recording. They observed that using fresh bedding in recording cages increased the number of 50 kHz vocalizations in all drug injection conditions, and this effect did not significantly decrease over test days. Although fresh bedding was used for each rat in this study, it was consistent across groups and drug conditions, and was done for every recording session, therefore it is unlikely that the observations made with regard to an increase in calls for both 0.25 μg of quinpirole and 7 μg of amphetamine are affected by bedding. In fact, it may enhance the finding that low doses of quinpirole other than the 0.25 μg dose had no effect on vocalization behaviour if the fresh bedding used during those recording sessions was unable to increase 50 kHz calls above saline levels. If fresh bedding was to increase 50 kHz calls in this case, it would have increased the magnitude of calls across all drug conditions because fresh bedding was used for each rat in each recording session. The work completed by Natusch and Schwarting (2010) is further evidence that environmental testing conditions when recording affective behaviours are important and are something that needs to be controlled for as best as possible.

Future Directions

Future research should look at the possibility of antagonizing the 50 kHz call response elicited by high doses of quinpirole using U-99194A maleate. Also, a D_2/D_3 receptor antagonist

"cocktail" could be injected as a pre-treatment to an optimal high dose of quinpirole which should then completely abolish calling rates. Also, using selective D_2 and/or D_3 autoreceptor antagonists may prove helpful in shaping a more exact mechanism of action at the receptor level for quinpirole.

With respect to 50 kHz vocalizations, much research is needed to look into the further sub-categorization of 50 kHz calls. Recent work completed by Wright et al., (2010) separated 50 kHz calls into fourteen sub-categories based on sonographic structure; however, to be useful for future research with respect to positive affective states, calls should be sub-categorized based on biological function. The work completed by Wright, et al., (2010) is encouraging because the calls with different sonographic structure that were categorized as "frequency-modulated other" in this thesis can possibly be further separated based on their functionality now that the possible categories have been defined. By accomplishing that task, it may aid in addiction, emotional, and motivational disorder research by allowing various types of drugs of abuse to be studied further based on the effects they have on emotion and motivation.

Conclusions

It is clear that quinpirole in the low dose range is acting at the D_3 receptor subtype, indicating that the D_3 dopamine receptor in the nucleus accumbens medial shell also contributes to 50 kHz vocalization behaviour. Also, low doses of quinpirole seem to be increasing positive affective states in the rat because the 0.25 μg dose significantly increased the frequency modulated type of 50 kHz calls, which are known to indicate an increase in positive affective states in rats (Panksepp & Burgdorf, 2000; 2003, Burgdorf et al., 2008; Ahrens et al., 2009). To

my knowledge, this is the first report of D_3 receptor involvement in 50 kHz USV production. Perhaps further research into the mechanisms underlying quinpirole's actions could help with a better understanding of emotional and motivational states in rats and may contribute to an animal model of emotional disorders with the ultimate goal being to better the lives of humans suffering from this type of abnormality.

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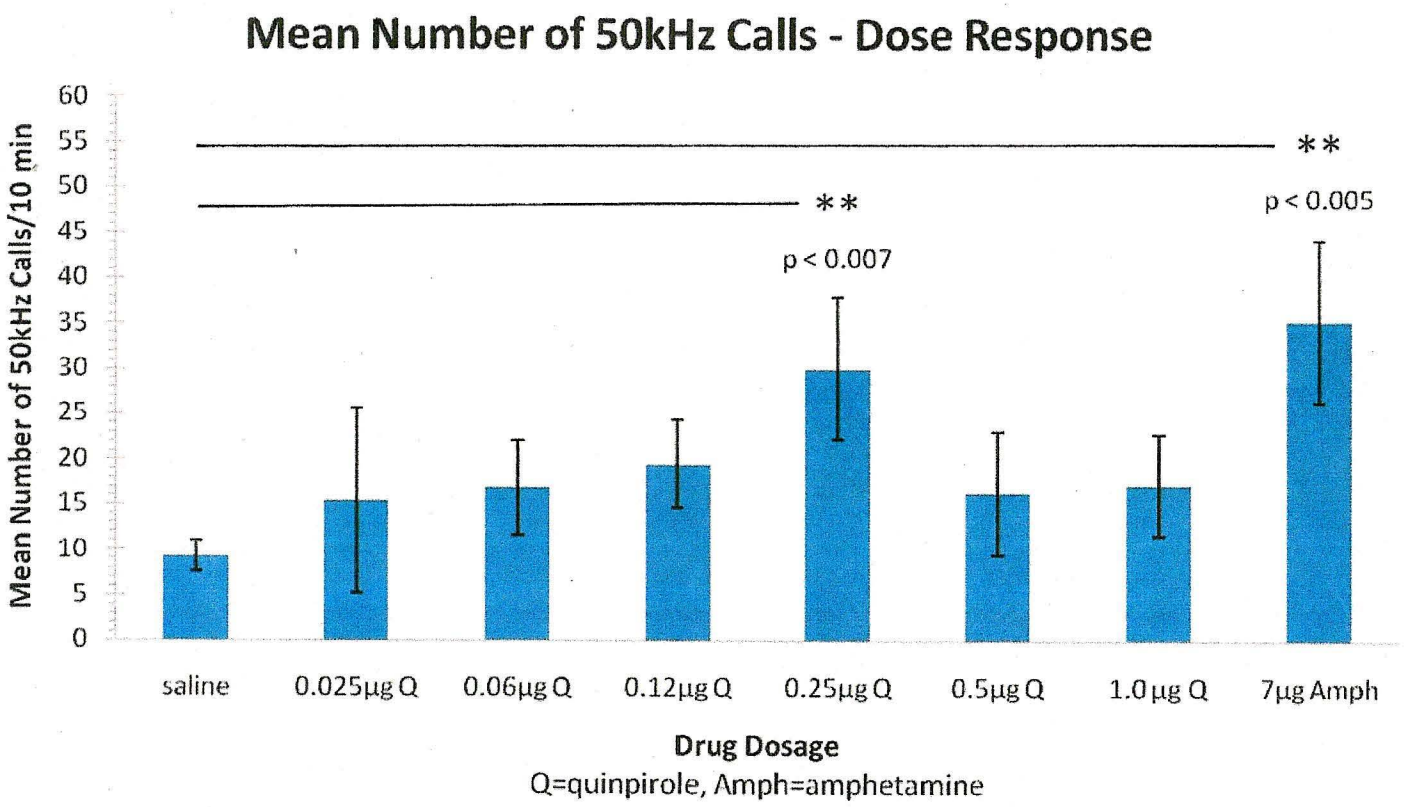


Figure 1. Full dose response for quinpirole at low doses (0.025 µg, 0.06 µg, 0.12 µg, 0.25 µg, 0.5 µg, and 1.0 µg) along with saline and the 7 µg amphetamine controls. The y-axis shows the mean number of 50 kHz vocalizations in ten minutes of recording. Error bars represent SEM. * indicates significance level $p < 0.05$ ** indicates significance level $p < .01$

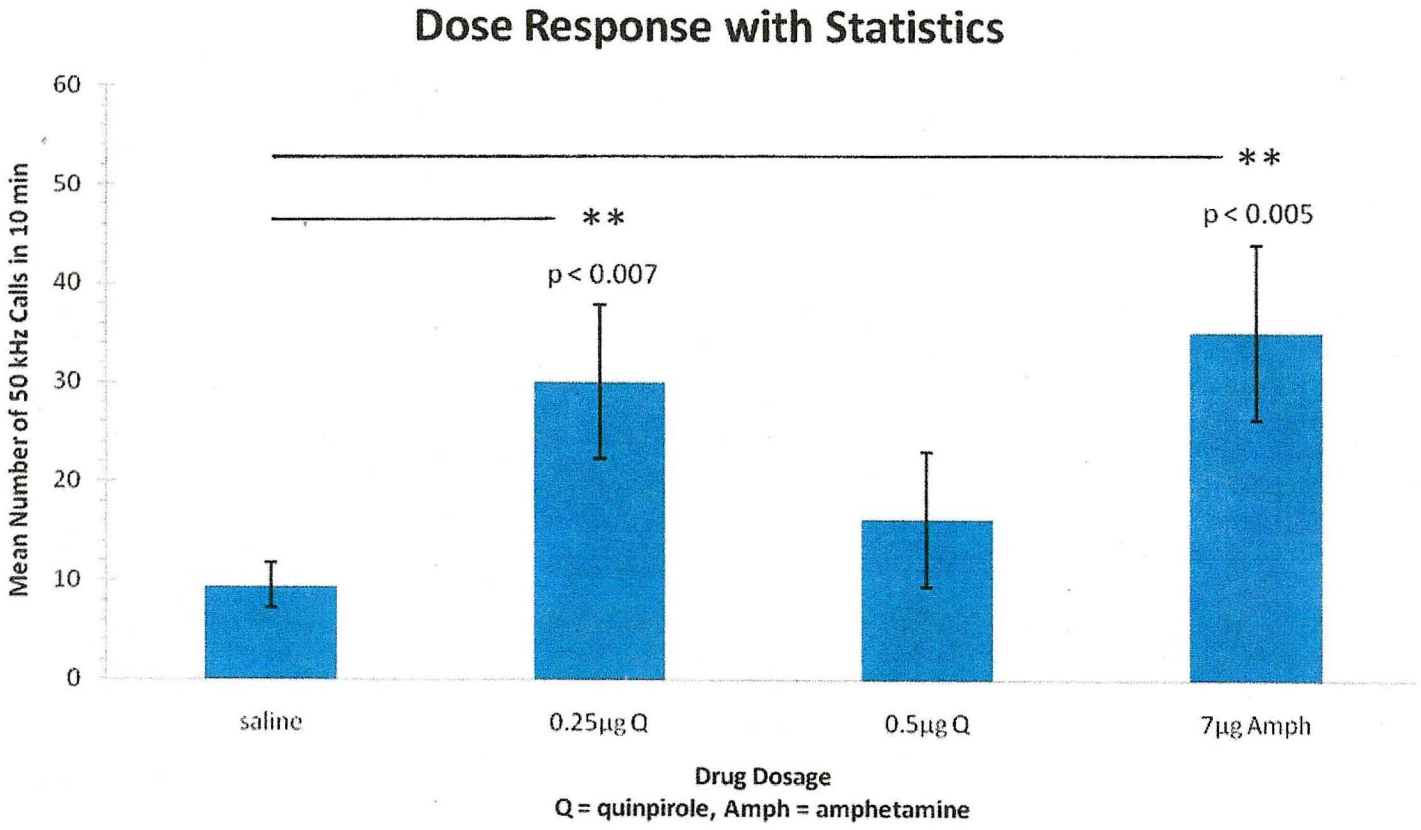


Figure 2. Mean number of 50 kHz calls within 10 minutes (groups 1 and 2) with statistical analyses. Saline and 7 µg amphetamine controls along with the 0.25 µg and 0.5 µg dosages of quinpirole. Statistical analyses were completed using the non-parametric Friedman ANOVA followed by the non-parametric Wilcoxon Signed Ranks Test.

Error bars represent SEM. * indicates significance level $p < 0.05$ ** indicates significance level $p < .01$

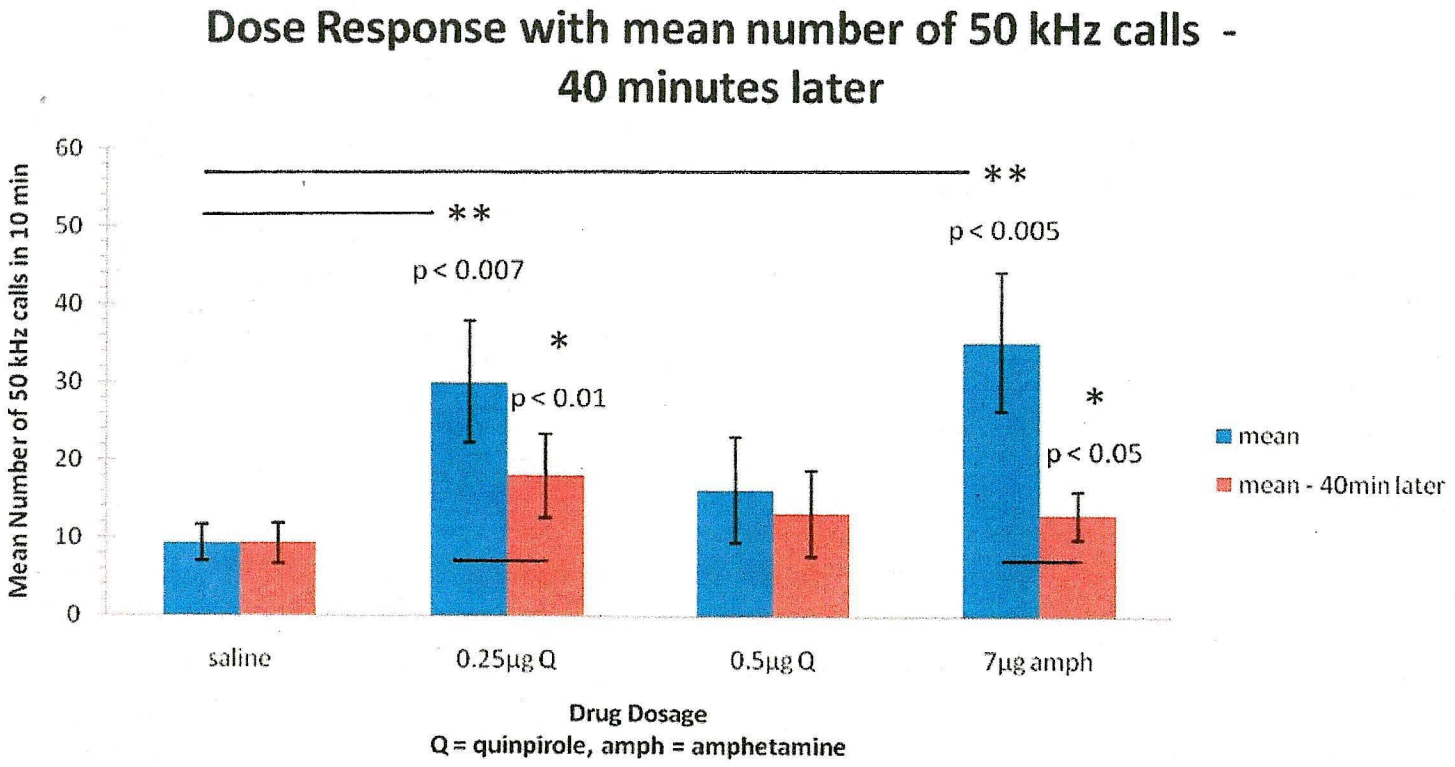


Figure 3. Mean number of 50 kHz calls within 10 minutes immediately following intracerebral injection and 40 minutes later with no additional injection. Statistical analyses were completed using the non-parametric Friedman ANOVA followed by the non-parametric Wilcoxon Signed Ranks Test. Error bars represent SEM. * indicates significance level p <0.05 ** indicates significance level p <.01

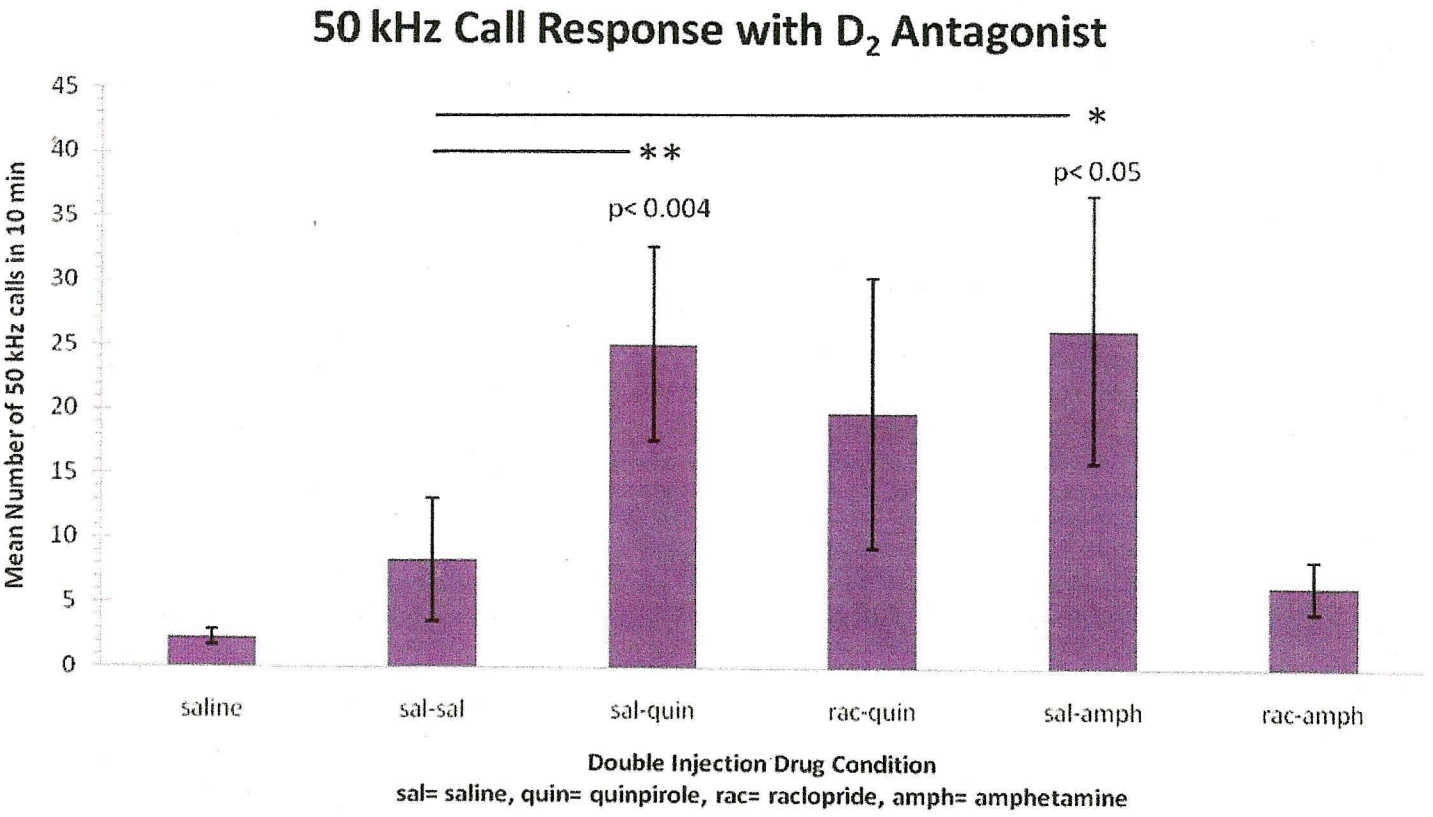


Figure 4. Mean number of 50 kHz calls in 10 minutes after double injections: pre-treatment with saline or D_2 antagonist raclopride. Psychostimulants used were 0.25 µg of quinpirole and 7 µg of amphetamine. Due to a relatively low N number of 11 rats, and the amount of inter-individual variability, sal-amph compared to rac-amph was not quite statistically significant although it was approaching significance ($p < 0.07$). Comparing rac-quin and rac-amph the D_2 antagonist response observed is not similar. Statistical analyses were completed using the non-parametric Friedman ANOVA followed by the non-parametric Wilcoxon Signed Ranks Test. Error bars represent SEM. * indicates significance level $p < 0.05$ ** indicates significance level $p < .01$

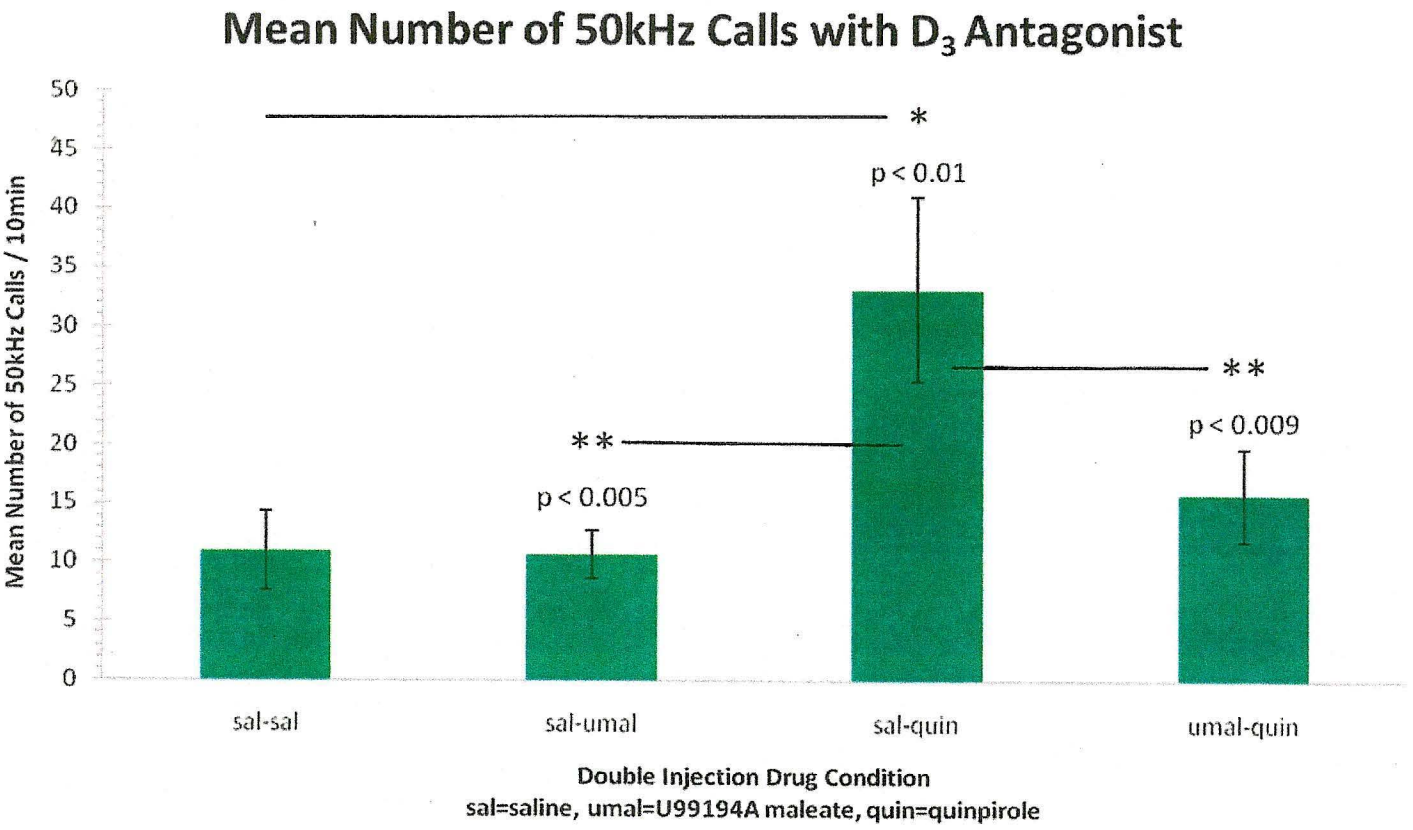


Figure 5. Mean number of 50 kHz calls in 10 minutes after unilateral double injections: pre-treatment with saline or D_3 antagonist U99194A maleate (equimolar to 0.25 µg quinpirole) followed by 0.25 µg quinpirole. Statistical analyses were completed using the non-parametric Friedman ANOVA followed by the non-parametric Wilcoxon Signed Ranks Test. Error bars represent SEM. * indicates significance level $p < 0.05$ ** indicates significance level $p < .01$

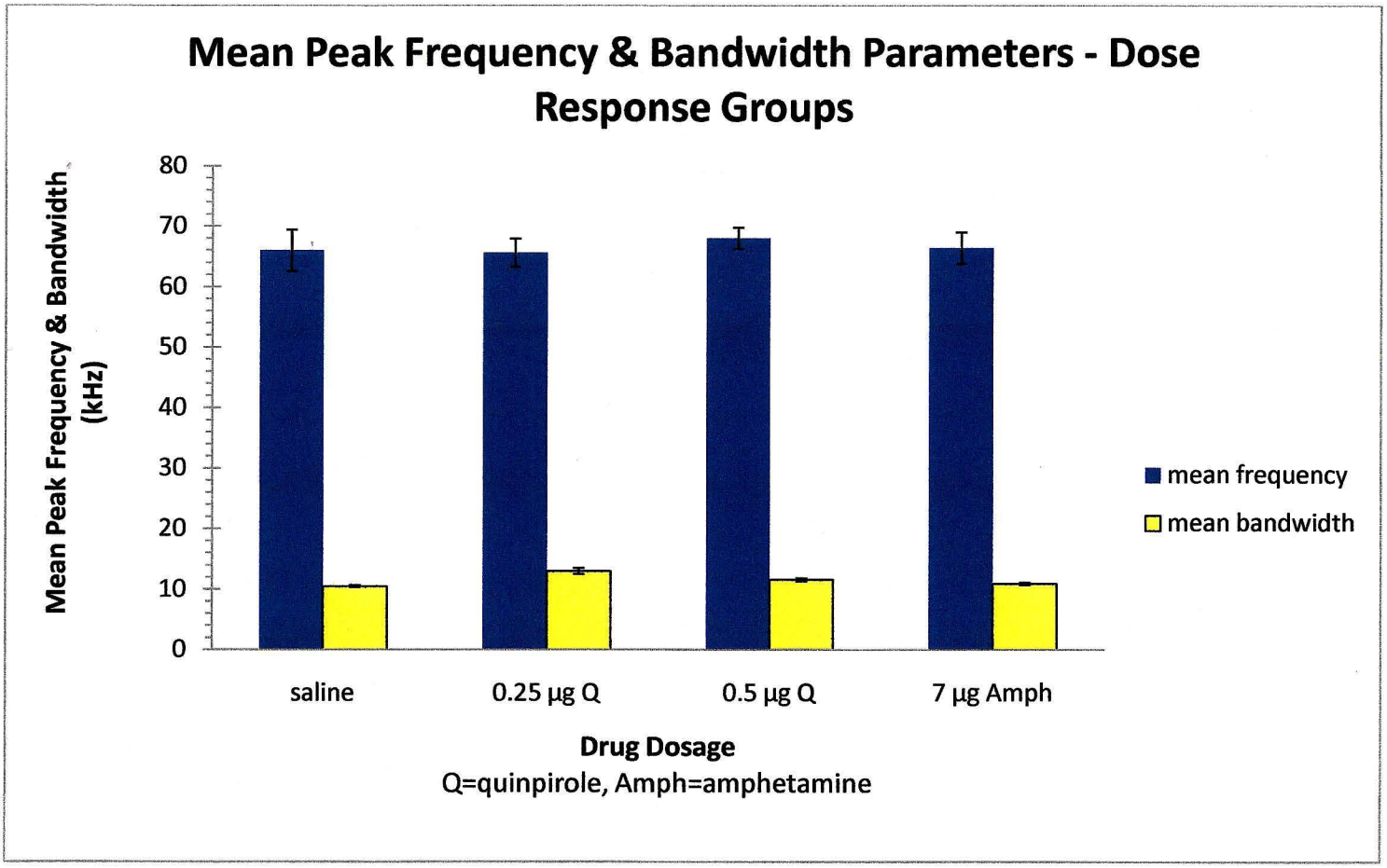


Figure 6. Mean call bandwidth and mean call peak frequencies (in kHz) of dose response groups (saline, 0.25 µg quinpirole, 0.5 µg quinpirole, 7 µg amphetamine). Error bars represent SEM.

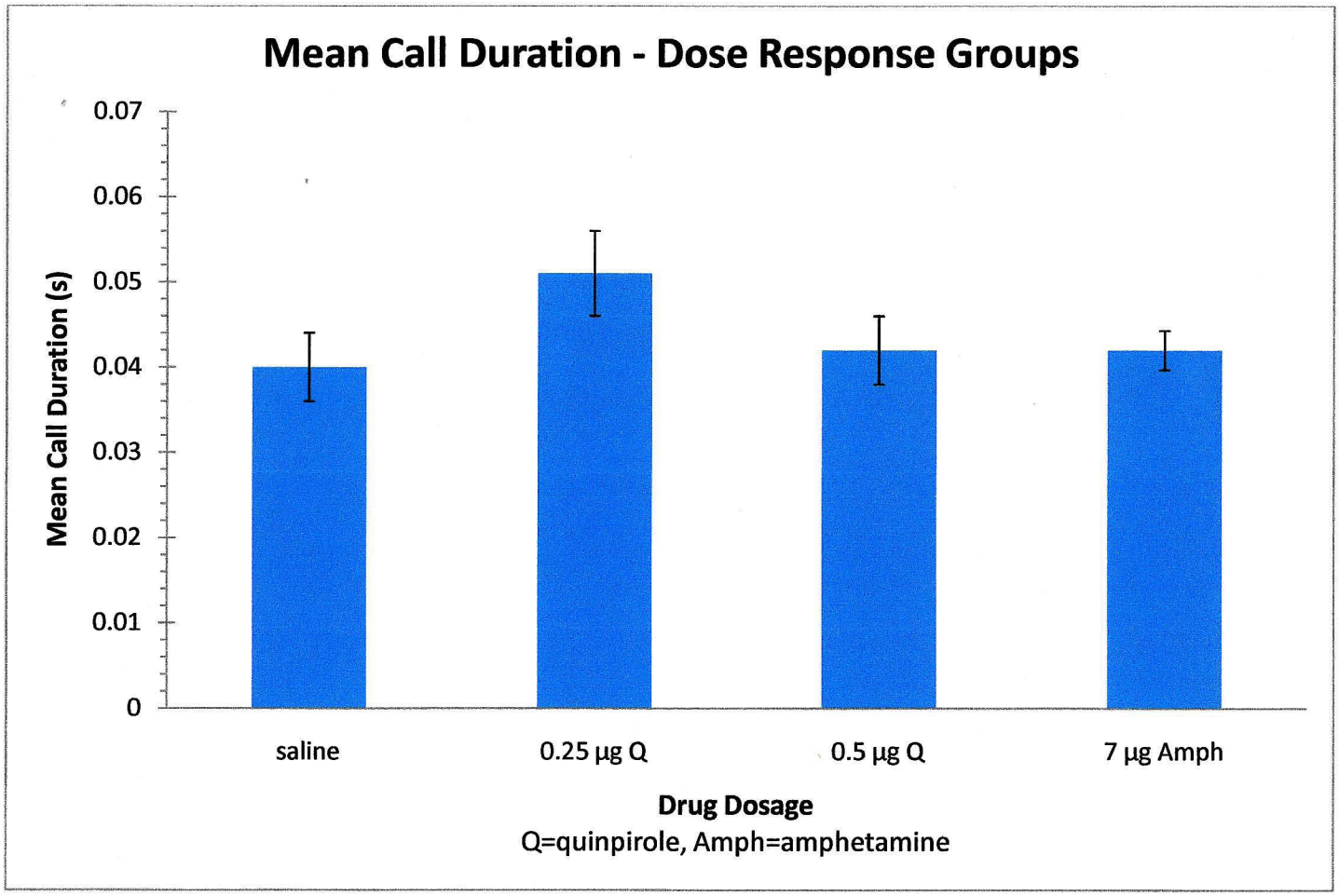


Figure 7. Mean call duration (in seconds) of dose response groups (saline, 0.25 µg quinpirole, 0.5 µg quinpirole, 7 µg amphetamine). Error bars represent SEM.

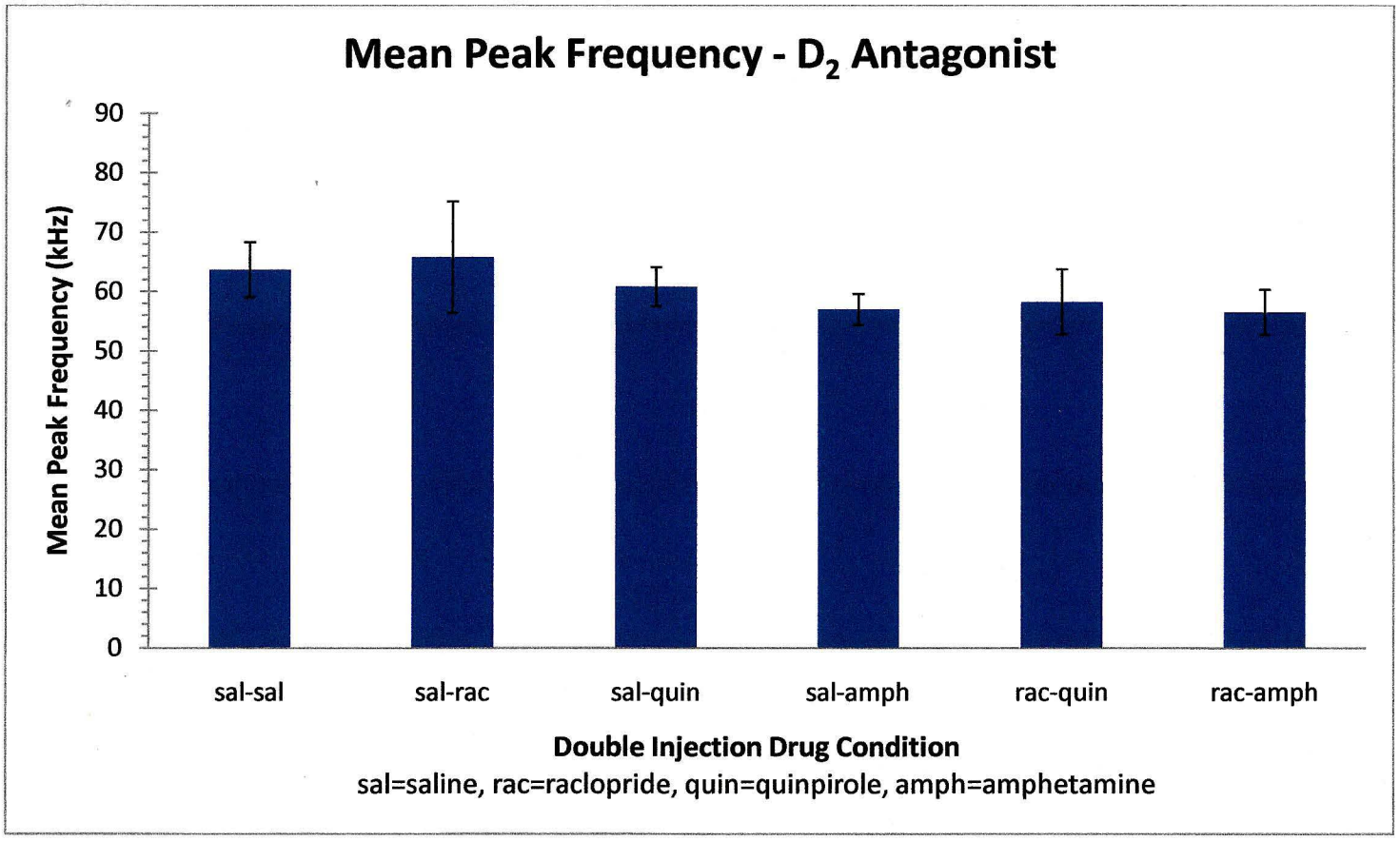


Figure 8. Mean call peak frequencies (in kHz) of D_2 antagonist double injection group using 0.25 µg quinpirole and 7 µg amphetamine. Error bars represent SEM.

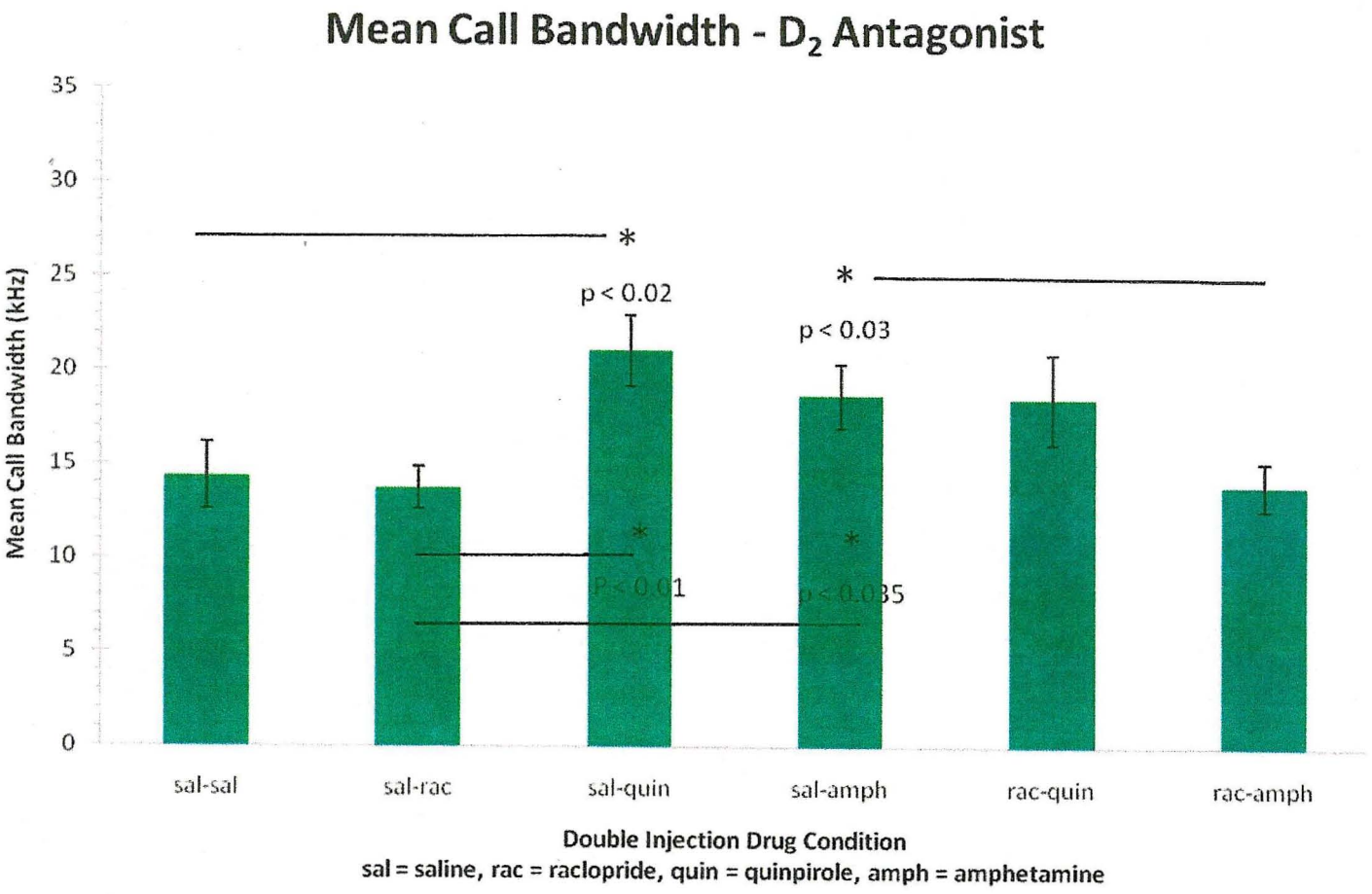


Figure 9. Mean call bandwidth (in kHz) of D_2 antagonist double injection group using 0.25 µg quinpirole and 7 µg amphetamine. Statistical analyses were completed using the non-parametric Friedman ANOVA followed by the non-parametric Wilcoxon Signed Ranks Test. Error bars represent SEM. * indicates significance level $p < 0.05$

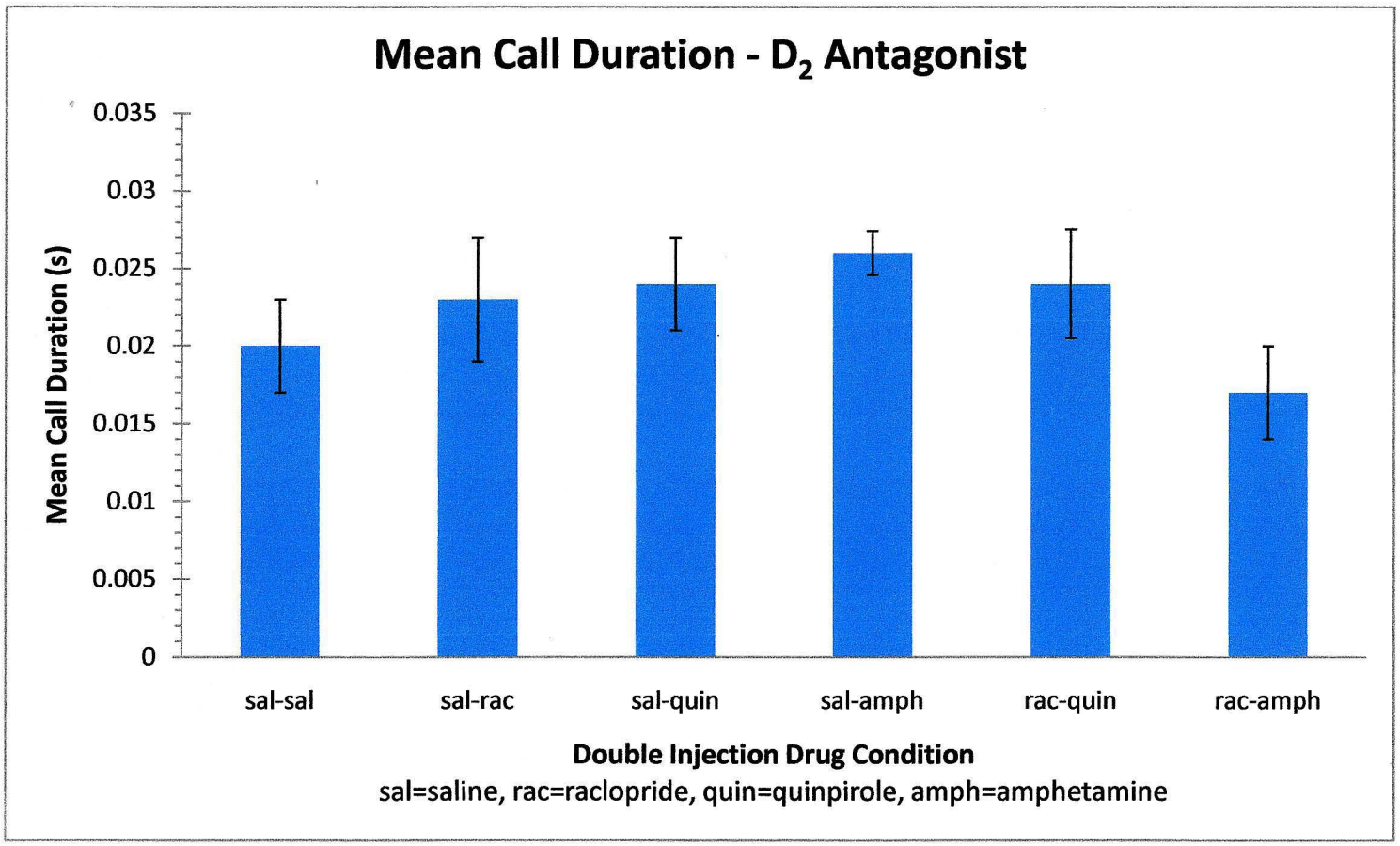


Figure 10. Mean call duration (in seconds) of D_2 antagonist double injection group using 0.25 µg quinpirole and 7 µg amphetamine. Error bars represent SEM.

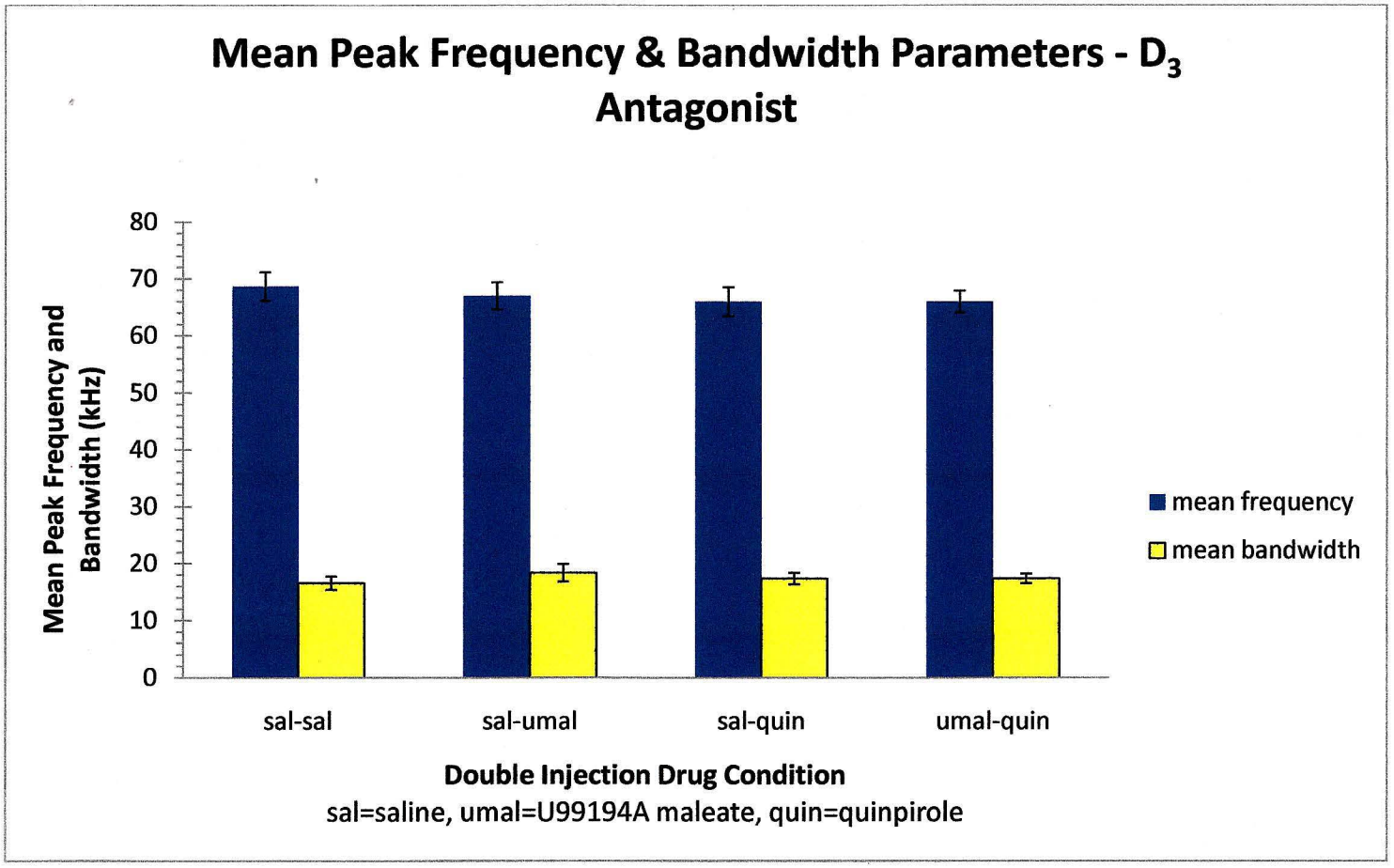


Figure 11. Mean call bandwidth and mean call peak frequencies (in kHz) of D_3 antagonist group using 0.25 µg quinpirole and 7 µg amphetamine. Error bars represent SEM.

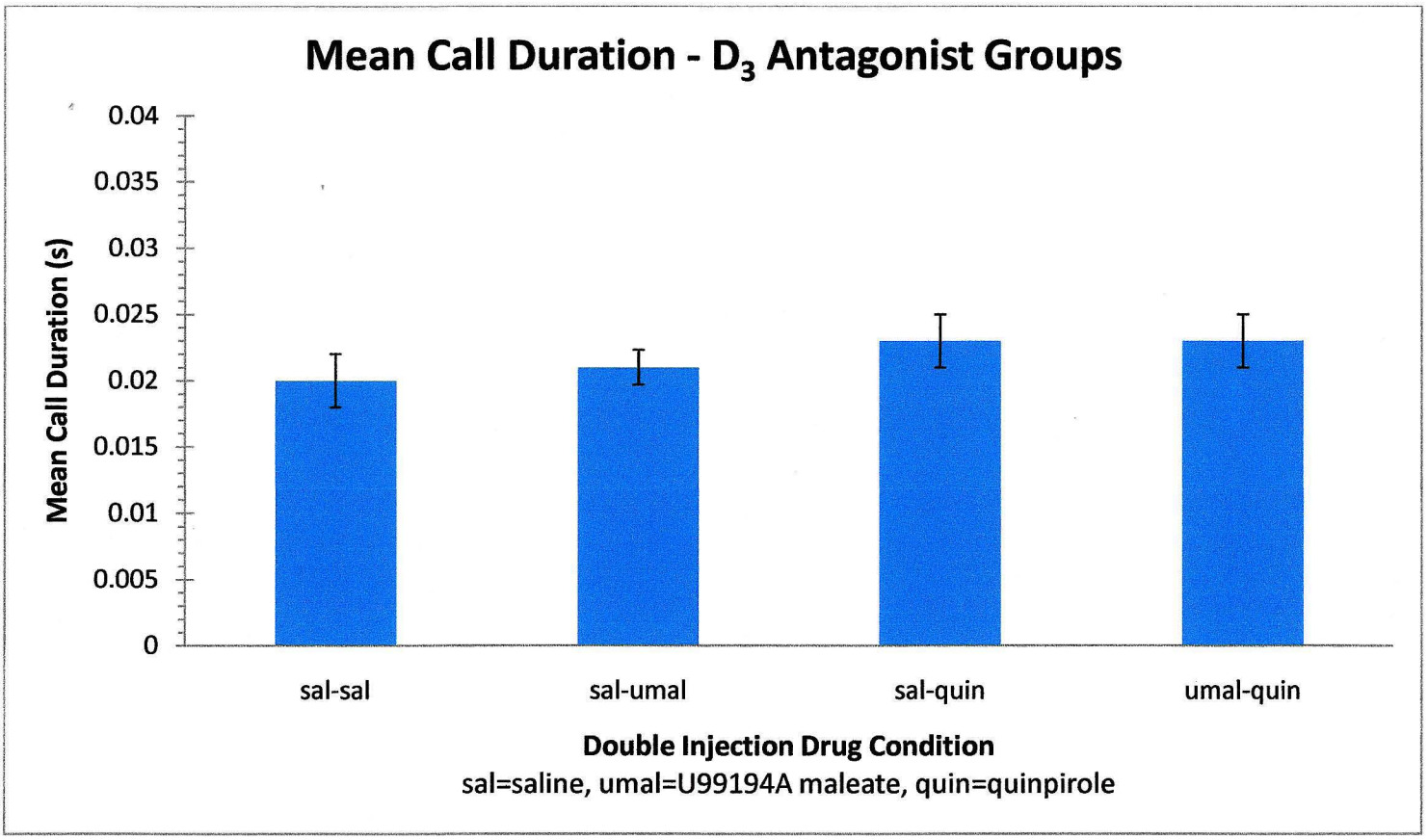


Figure 12. Mean call duration (in seconds) of D_3 antagonist group using 0.25 µg quinpirole and 7 µg amphetamine. Error bars represent SEM.

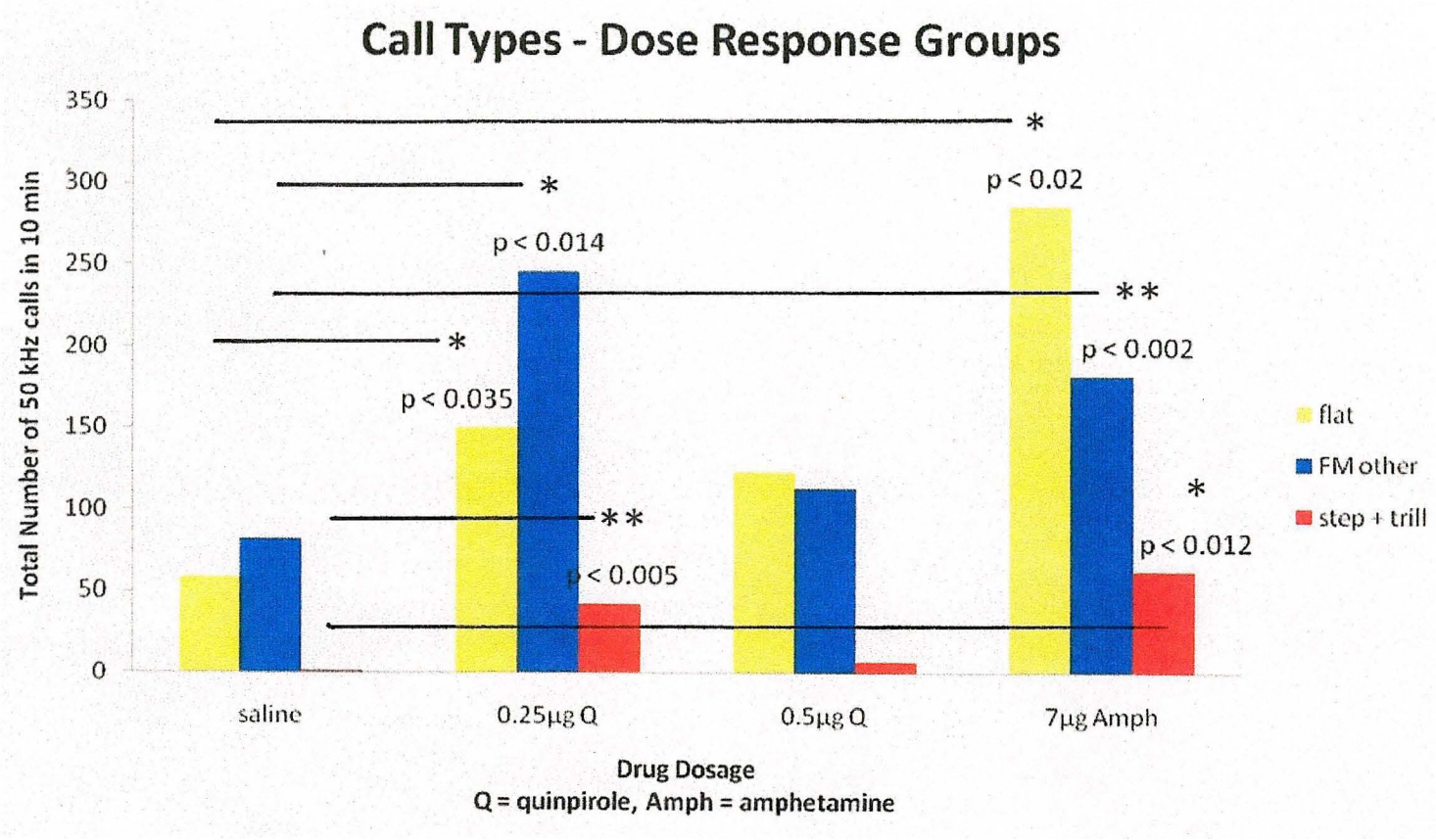


Figure 13. Total numbers of sonographic call types (listed on margin) observed in each drug condition for dose response groups used for statistical analysis (saline, 0.25 µg quinpirole, 0.5 µg quinpirole, 7 µg amphetamine). Statistical analyses were completed using the non-parametric Friedman ANOVA followed by the non-parametric Wilcoxon Signed Ranks Test. * indicates significance level $p < 0.05$ ** indicates significance level $p < .01$

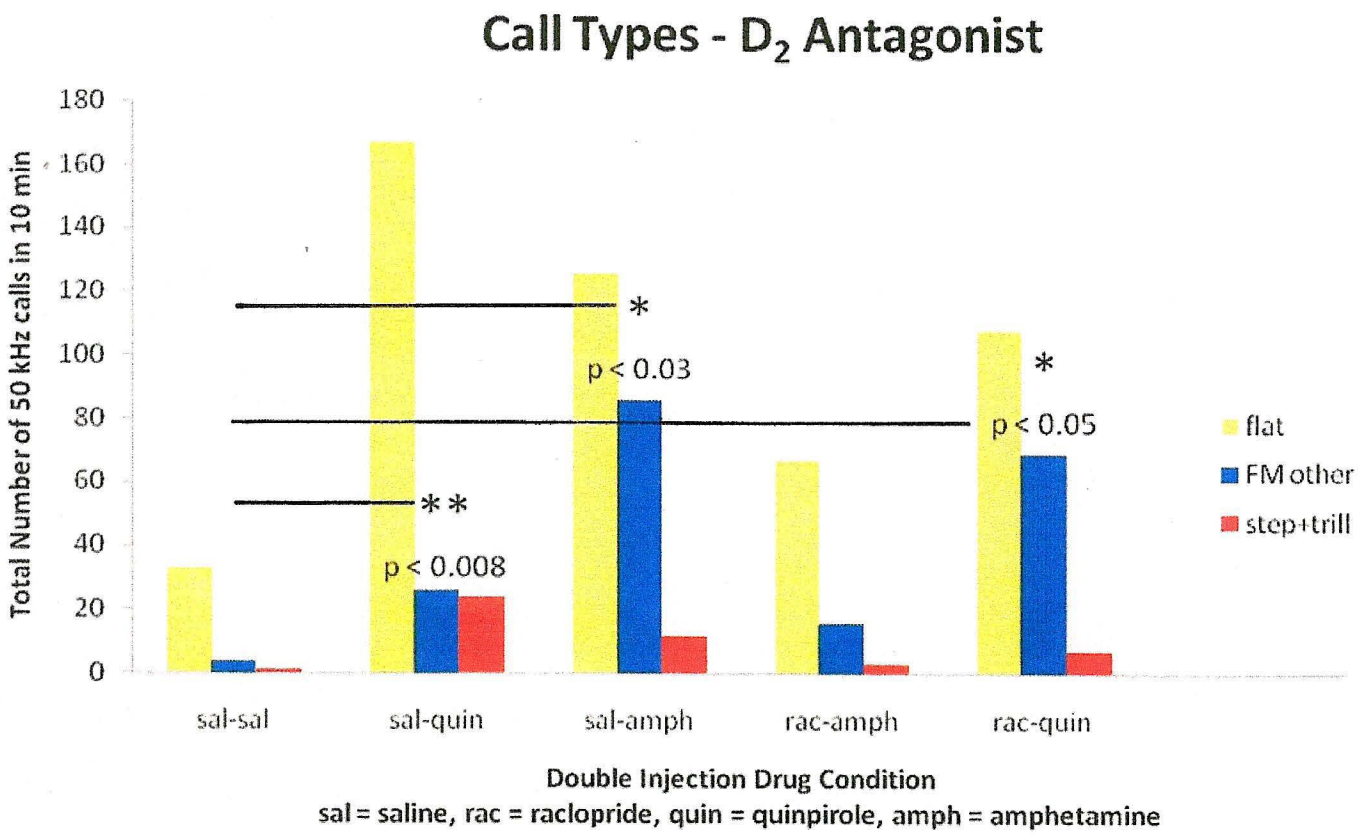


Figure 14. Total numbers of sonographic call types (listed on margin) observed in each drug condition for D_2 antagonist double injections pre-treated with saline or raclopride followed by the 0.25 µg dose of quinpirole or the 7 µg amphetamine dose. Statistical analyses were completed using the non-parametric Friedman ANOVA followed by the non-parametric Wilcoxon Signed Ranks Test. * indicates significance level $p < 0.05$ ** indicates significance level $p < .01$

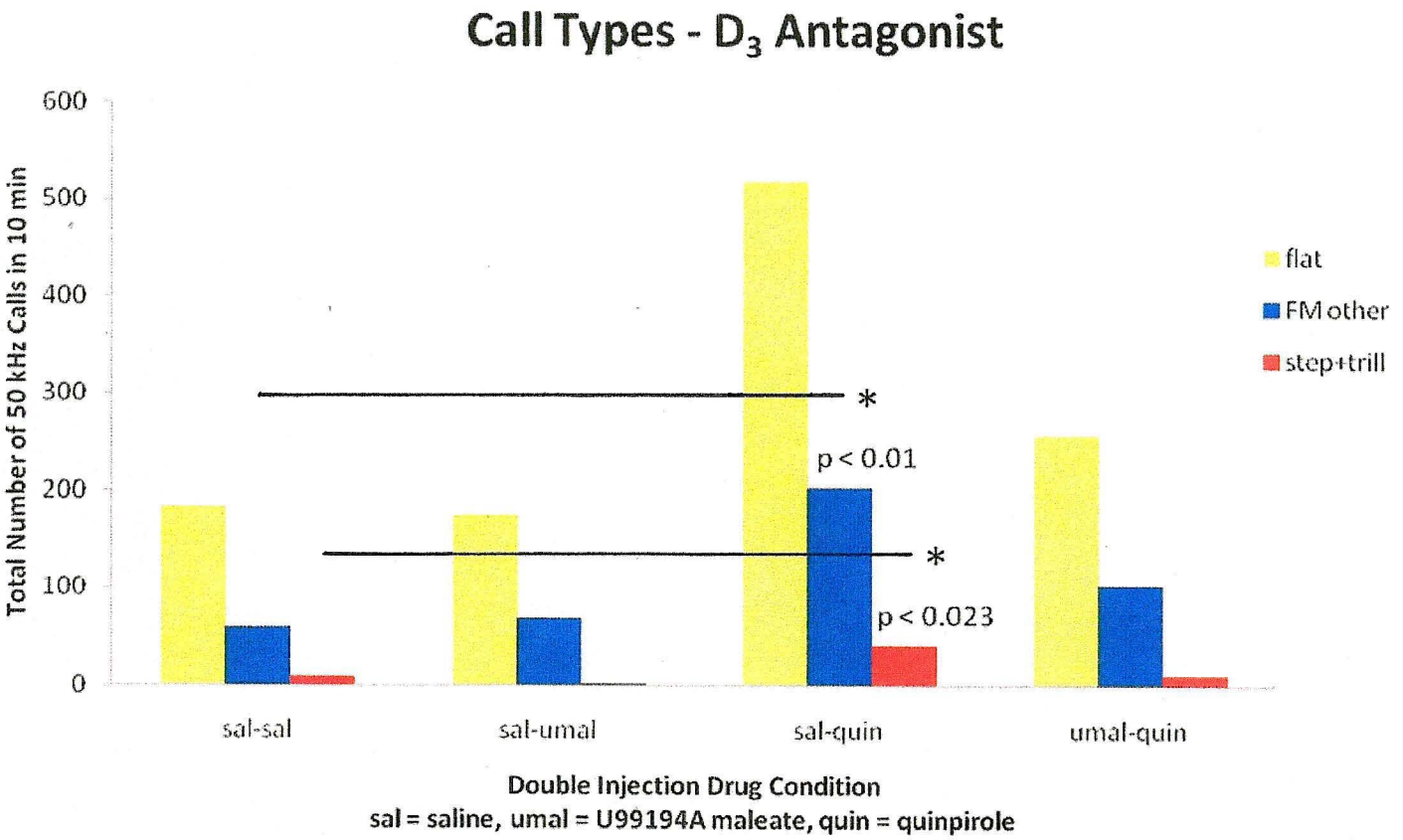


Figure 15. Total numbers of sonographic call types (listed on margin) observed in each drug condition for D_3 antagonist double injections pre-treated with saline or U99194A maleate followed by the 0.25 µg dose of quinpirole. Statistical analyses were completed using the non-parametric Friedman ANOVA followed by the non-parametric Wilcoxon Signed Ranks Test. * indicates significance level $p < 0.05$ ** indicates significance level $p < .01$

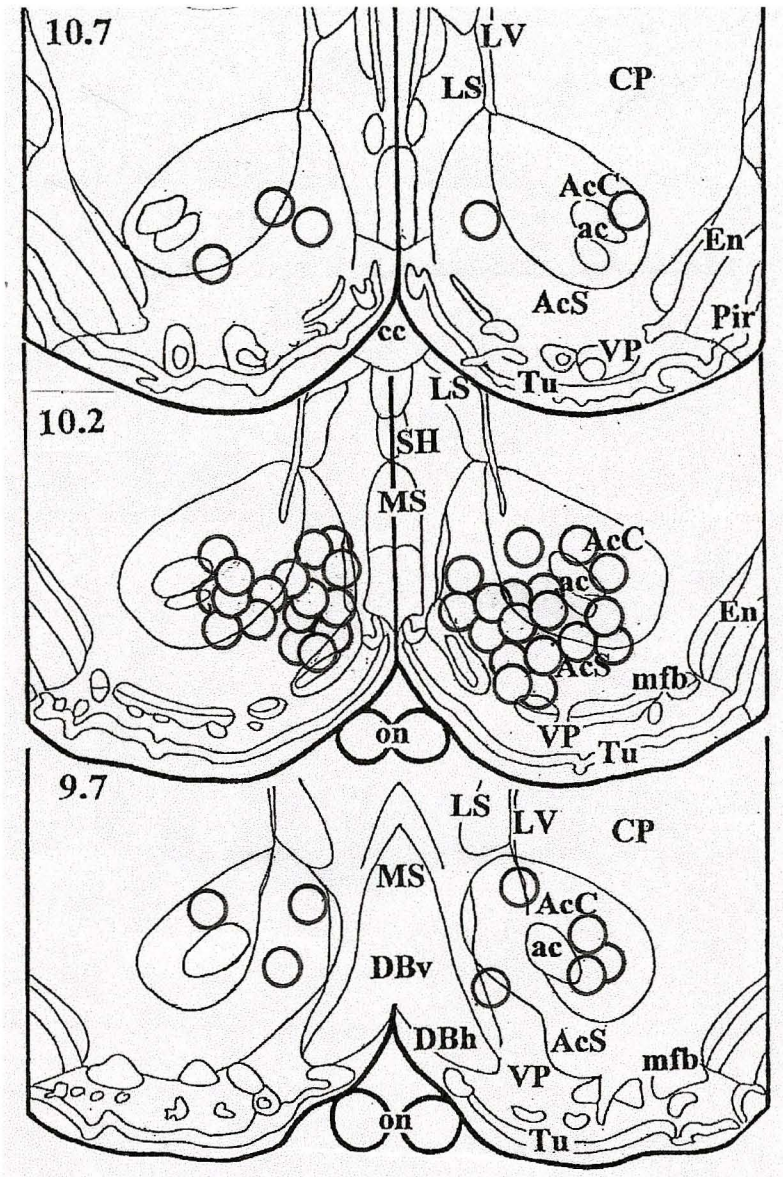


Figure 16. Anatomical localization of intracerebral injection sites for dose response groups (groups 1-3) on coronal sections from the atlas of Paxinos and Watson (1986). All injections were from 10.7-9.7 mm from lambda placing them in the medial nucleus accumbens shell.

List of Abbreviations: **AcC** = nucleus accumbens core, **ac** = anterior commissure, **AcS** = nucleus accumbens shell, **CP** = caudate putamen, **LS** = lateral septal nucleus, **LV** = lateral ventricle, **mfb** = medial forebrain bundle, **VP** = ventral pallidum, **Tu** = olfactory tubercule, **on** = optic nerve, **DBh** = nu horizontal limb diagonal band, **DBv** = nu vertical limb diagonal band, **MS** = medial septal nucleus

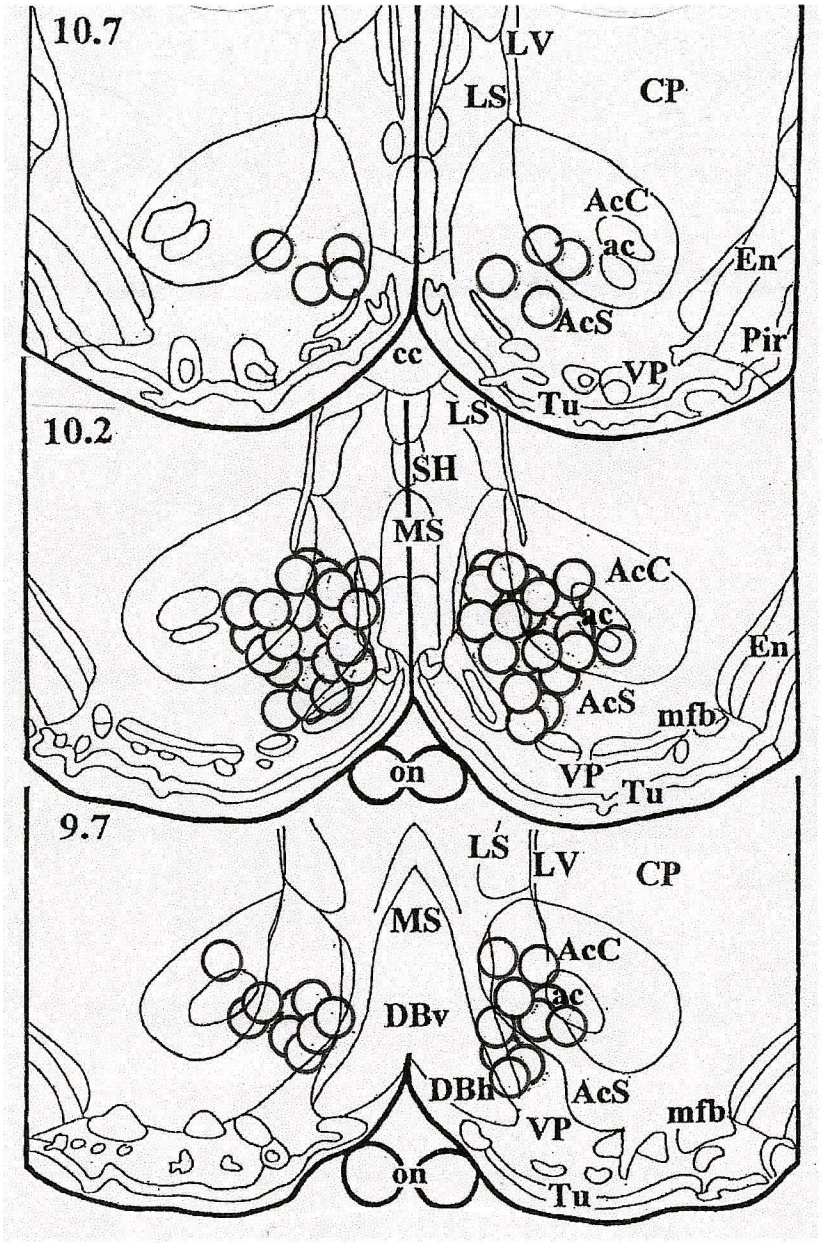
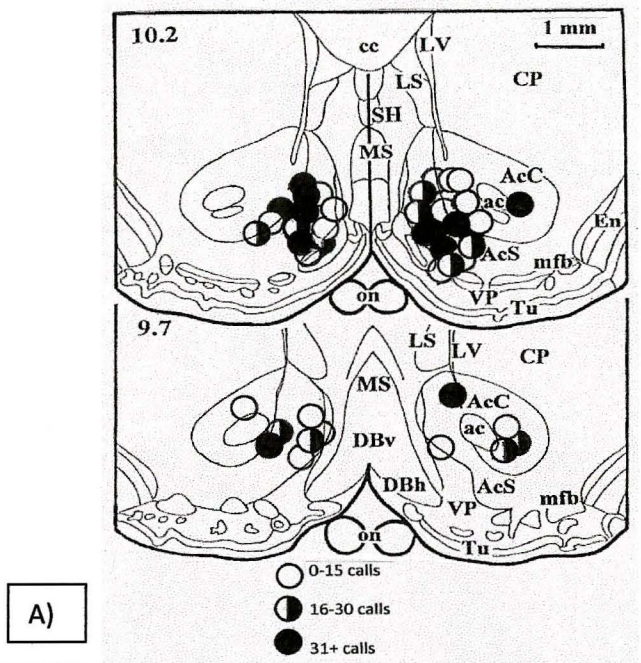
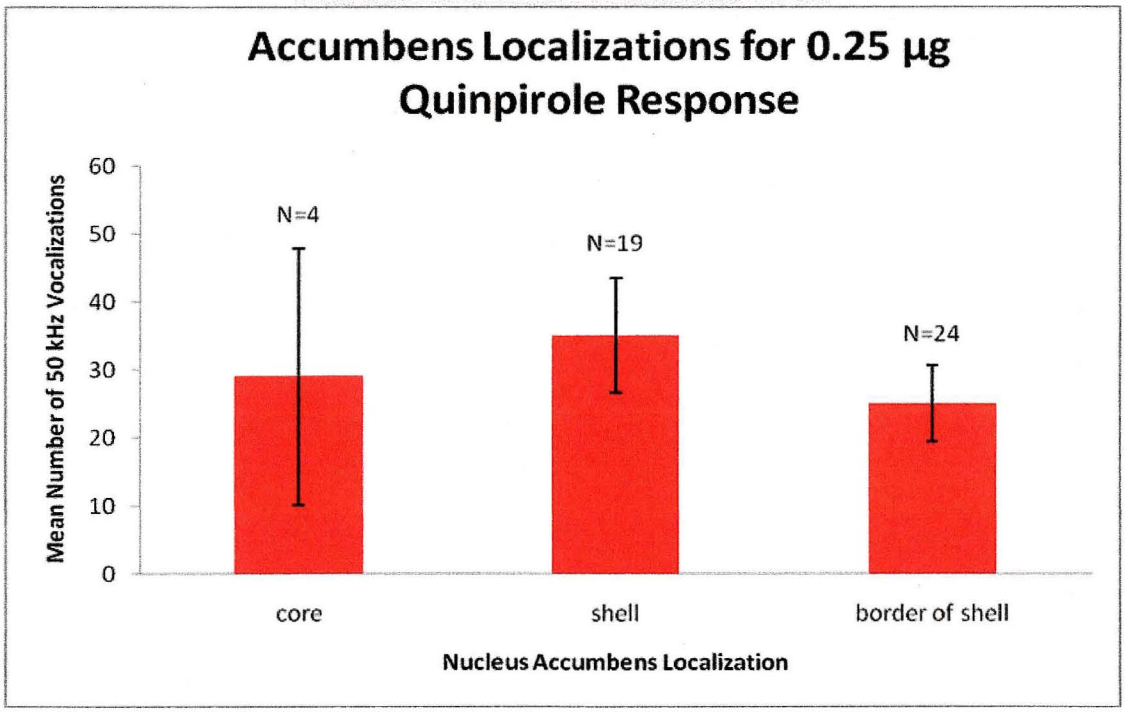


Figure 17. Anatomical localization of intracerebral injection sites for double injection groups (groups 4-6) on coronal sections from the atlas of Paxinos and Watson (1986). All injections were from 10.7-9.7 mm from lambda placing them in the medial nucleus accumbens shell. Refer to Figure 16 for abbreviations.

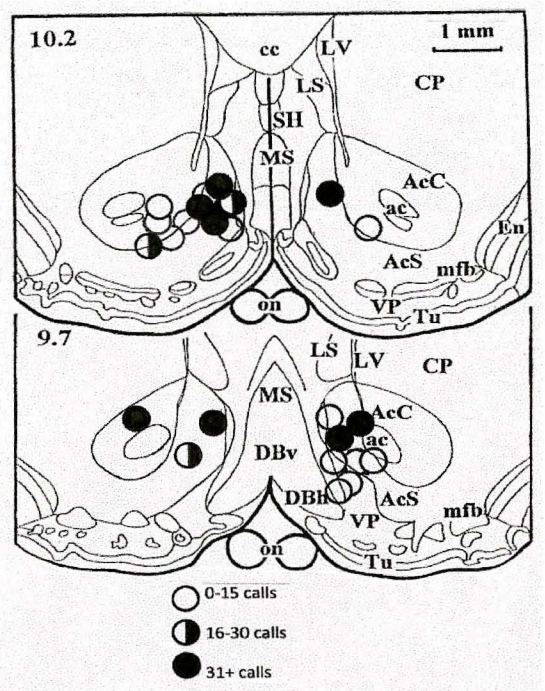


A)

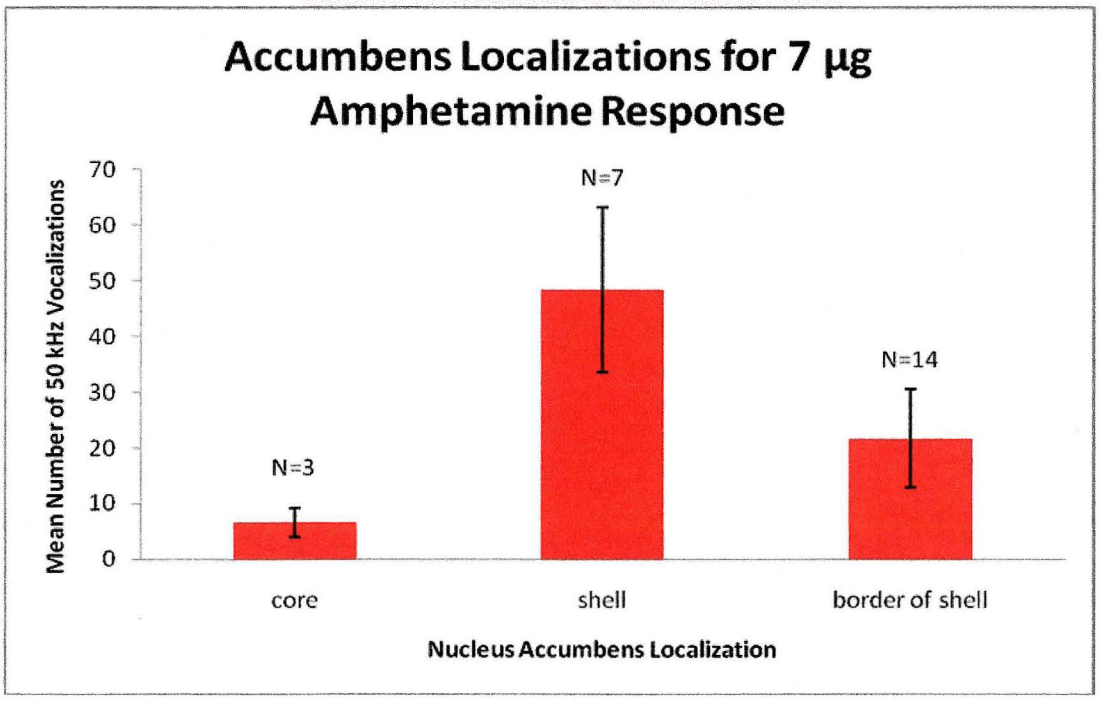


B)

Figure 18. Mapping of 50 kHz call magnitude elicited by 0.25 µg injections of quinpirole. A) Shows localization of injection sites with symbols indicating the number of calls induced. B) Shows a summary of the mean number of calls per anatomical localization. Due to a small N number in the core localization, statistics were not able to be performed. However, it is apparent that the majority of high vocalizing rats were injected in the nucleus accumbens shell. Error bars represent SEM. Refer to Figure 16 for abbreviations.



A)



B)

Figure 19. Mapping of 50 kHz call magnitude elicited by 7 µg injections of amphetamine. A) Shows localization of injection sites with symbols indicating the number of calls induced. B) Shows a summary of the mean number of calls per anatomical localization. Due to a small N number in the core localization, statistics were not able to be performed. However, it is apparent that the majority of high vocalizing rats were injected in the nucleus accumbens shell. Error bars represent SEM. Refer to Figure 16 for abbreviations.

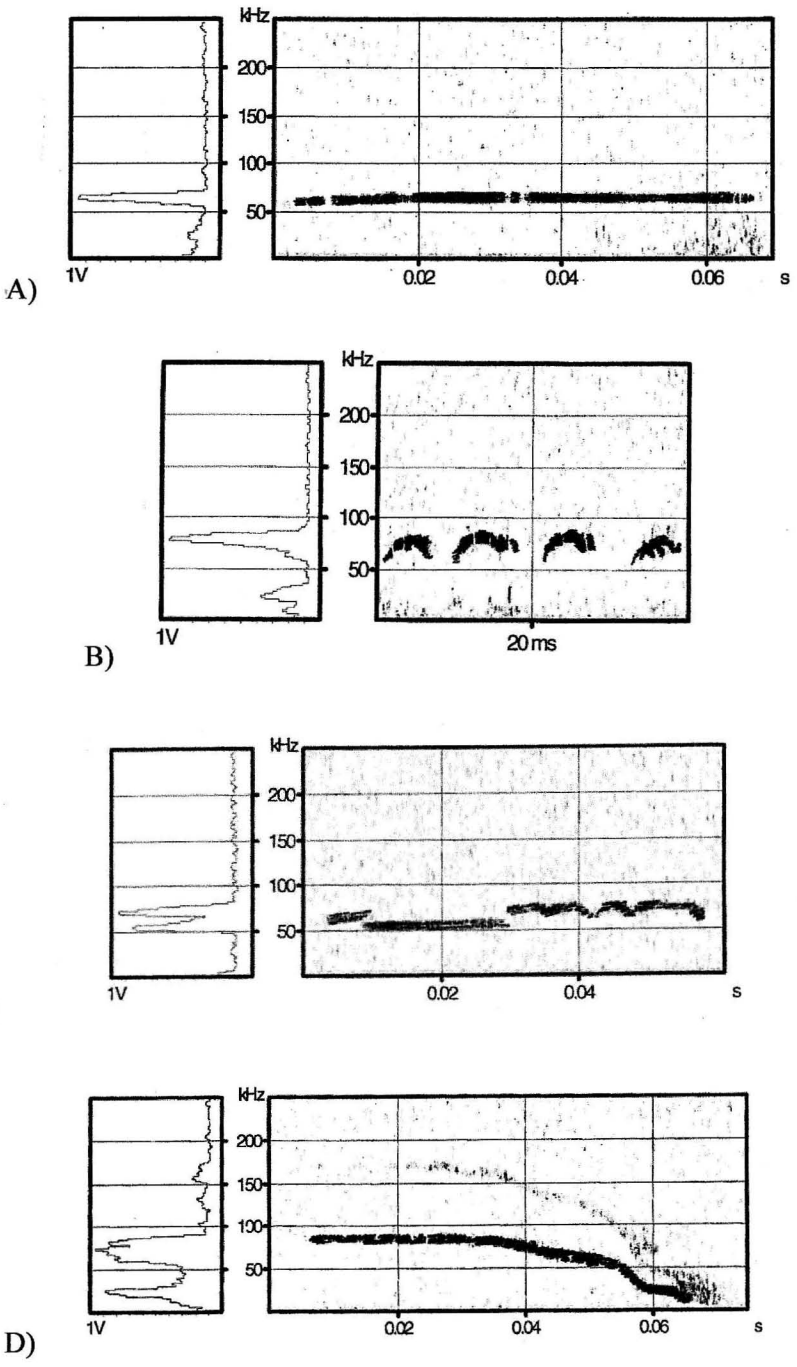


Figure 20. A few samples of different 50 kHz vocalizations elicited by 0.25 μg injection of quinpirole. Each figure includes a spectrogram (on the right hand side), and a power spectrum (on the left hand side). A) Flat 50 kHz vocalization B) Trill 50 kHz vocalization C) Step-trill 50 kHz vocalization D) FM other (frequency modulated other) 50 kHz vocalization. Frequency is in kHz, time is in ms, and power is on relative values.

Appendix A

Abbreviations

Quin = Quinpirole

Amph = Amphetamine

MWQ = male, wistar, quinpirole

UMAL = U99194A Maleate

RAC = Raclopride

Dose Response Groups Intracerebral Injection Schedule and Drug Dosage					
Rat I.D. ***Group #1***	**Injection 1 – Right Cannula**	**Injection 2 – Right Cannula**	**Injection 3 – Right Cannula**	**Injection 4 – Right Cannula**	**Injection 5 – Left Cannula**
MWQ1	0.25µg Quin	saline	0.5µg Quin	1.0µg Quin	7µg Amph
MWQ2	saline	0.25µg Quin	1.0µg Quin	0.5µg Quin	7µg Amph
MWQ3	0.25µg Quin	saline	0.5µg Quin	1.0µg Quin	7µg Amph
MWQ4	saline	0.25µg Quin	1.0µg Quin	0.5µg Quin	7µg Amph
MWQ5	0.25µg Quin	saline	0.5µg Quin	1.0µg Quin	7µg Amph
MWQ6	saline	0.25µg Quin	1.0µg Quin	0.5µg Quin	7µg Amph
MWQ7	0.25µg Quin	saline	0.5µg Quin	1.0µg Quin	7µg Amph
MWQ8	1.0µg Quin	0.5µg Quin	0.25µg Quin	saline	7µg Amph
MWQ9	0.5µg Quin	1.0µg Quin	saline	0.25µg Quin	7µg Amph
MWQ10	1.0µg Quin	0.5µg Quin	0.25µg Quin	saline	7µg Amph
MWQ11	0.5µg Quin	1.0µg Quin	saline	0.25µg Quin	7µg Amph
MWQ12	1.0µg Quin	0.5µg Quin	0.25µg Quin	saline	7µg Amph
MWQ13	0.5µg Quin	1.0µg Quin	saline	0.25µg Quin	7µg Amph
MWQ14	1.0µg Quin	0.5µg Quin	0.25µg Quin	saline	7µg Amph
Group #2	**Injection 1 – Right Cannula**	**Injection 2 – Right Cannula**	**Injection 3 – Right Cannula**	**Injection 4 – Right Cannula**	**Injection 5 – Left Cannula**
MWQ15	saline	0.25µg Quin	0.12µg Quin	0.5µg Quin	7µg Amph
MWQ16	0.25µg Quin	saline	0.5µg Quin	0.12µg Quin	7µg Amph
MWQ17	saline	0.25µg Quin	0.12µg Quin	0.5µg Quin	7µg Amph
MWQ18	0.25µg Quin	saline	0.5µg Quin	0.12µg Quin	7µg Amph
MWQ19	saline	0.25µg Quin	0.12µg Quin	0.5µg Quin	7µg Amph
MWQ20	0.25µg Quin	saline	0.5µg Quin	0.12µg Quin	7µg Amph
MWQ21	saline	0.25µg Quin	0.12µg Quin	0.5µg Quin	7µg Amph
MWQ22	0.25µg Quin	saline	0.5µg Quin	0.12µg Quin	7µg Amph
MWQ23	0.12µg Quin	0.5µg Quin	0.25µg Quin	saline	7µg Amph
MWQ24	0.5µg Quin	0.12µg Quin	saline	0.25µg Quin	7µg Amph
MWQ25	0.12µg Quin	0.5µg Quin	0.25µg Quin	saline	7µg Amph
MWQ26	0.5µg Quin	0.12µg Quin	saline	0.25µg Quin	7µg Amph

MWQ27	0.12µg Quin	0.5µg Quin	0.25µg Quin	saline	7µg Amph
MWQ28	0.5µg Quin	0.12µg Quin	saline	0.25µg Quin	7µg Amph
MWQ29	0.12µg Quin	0.5µg Quin	0.25µg Quin	saline	7µg Amph
MWQ30	0.5µg Quin	0.12µg Quin	saline	0.25µg Quin	7µg Amph
Group #3	**Injection 1 – Right Cannula**	**Injection 2 – Right Cannula**	**Injection 3 – Right Cannula**	**Injection 4 – Right Cannula**	
MWQ31	saline	0.06 ug Quin	1.0 ug Quin	0.025 ug Quin	
MWQ32	0.06 ug Quin	Saline	0.025 ug Quin	1.0 ug Quin	
MWQ33	saline	0.06 ug Quin	1.0 ug Quin	0.025 ug Quin	
MWQ34	0.06 ug Quin	Saline	0.025 ug Quin	1.0 ug Quin	
MWQ35	Saline	0.06 ug Quin	1.0 ug Quin	0.025 ug Quin	
MWQ36	0.06 ug Quin	Saline	0.025 ug Quin	1.0 ug Quin	
MWQ37	1.0 ug Quin	0.025 ug Quin	Saline	0.06 ug Quin	
MWQ38	0.025 ug Quin	1.0 ug Quin	0.06 ug Quin	Saline	
MWQ39	1.0 ug Quin	0.025 ug Quin	Saline	0.06 ug Quin	
MWQ40	0.025 ug Quin	1.0 ug Quin	0.06 ug Quin	Saline	
MWQ41	1.0 ug Quin	0.025 ug Quin	Saline	0.06 ug Quin	
MWQ42	0.025 ug Quin	1.0 ug Quin	0.06 ug Quin	saline	

Injection Schedule Group #4 – Double injections

All QUIN = 0.25 µg dose of Quinpriole

All RAC = equimolar dose of raclopride to either quinpirole or amphetamine

All AMPH = 7 µg dose of amphetamine

All UMAL (D3 antagonist) = dose equimolar to quinpirole

Left Cannula	Rat 43, 50 & 55	Rat 45, 46 & 56	Rat 47, 48 & 57	Rat 49 & 44	Rat 51 & 52	Rat 53 & 54
Injection 1	SAL QUIN	RAC QUIN	SAL SAL	SAL SAL	RAC QUIN	SAL QUIN
Injection 3	RAC QUIN	SAL QUIN	SAL QUIN	RAC QUIN	SAL SAL	SAL SAL
Injection 5	SAL SAL	SAL SAL	RAC QUIN	SAL QUIN	SAL QUIN	RAC QUIN

Right Cannula	Rat 43, 50 & 55	Rat 45, 46 & 56	Rat 47, 48 & 57	Rat 49 & 44	Rat 51 & 52	Rat 53 & 54
Injection 2	SAL-AMPH	RAC-AMPH	SAL -RAC	SAL-RAC	RAC-AMPH	SAL-AMPH
Injection 4	RAC-AMPH	SAL-AMPH	SAL-AMPH	RAC-AMPH	SAL-RAC	SAL-RAC
Injection 6	SAL-RAC	SAL-RAC	RAC-AMPH	SAL-AMPH	SAL-AMPH	RAC-AMPH

Group 5 Injection Plan

Right Cannula	Rat 58, 63, 68, 73	Rat 59, 64, 69	Rat 60, 65, 70	Rat 61, 66, 71	Rat 62, 67, 72
Injection 1	SAL-UMAL	UMAL-QUIN	SAL-QUIN	SAL-SAL	SAL-UMAL
Injection 3	UMAL-QUIN	SAL-UMAL	SAL-SAL	UMAL-QUIN	SAL-QUIN

Left Cannula	Rat 58, 63, 68, 73	Rat 59, 64, 69	Rat 60, 65, 70	Rat 61, 66, 71	Rat 62, 67, 72
Injection 2	SAL-QUIN	SAL-SAL	UMAL-QUIN	SAL-UMAL	SAL-SAL
Injection 4	SAL-SAL	SAL-QUIN	SAL-UMAL	SAL-QUIN	UMAL-QUIN

Group 6 Injection Plan

Right Cannula	Rat 74, 75, 82	Rat 76, 77, 83	Rat 78, 79, 84	Rat 80, 81, 85
Injection 1	SAL-UMAL	SAL-SAL	UMAL-QUIN	SAL-QUIN
Injection 3	SAL-SAL	SAL-UMAL	SAL-QUIN	UMAL-QUIN

Left Cannula	Rat 74, 75, 82	Rat 76, 77, 83	Rat 78, 79, 84	Rat 80, 81, 85
Injection 2	UMAL-QUIN	SAL-QUIN	SAL-SAL	SAL-UMAL
Injection 4	SAL-QUIN	UMAL-QUIN	SAL-UMAL	SAL-SAL

Appendix B